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(54) Title: BACTERIAL STRAINS, GENES AND ENZYMES FOR CONTROL OF BACTERIAL DISEASES BY QUENCHING QUORUM-SENSING SIGNALS

(57) Abstract: The present invention relates to isolated nucleic acid molecules encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, and to expression vectors and transformed plant and animal cells comprising the same. The proteins encoded by these nucleic acid molecules provide to a susceptible plant or animal increased resistance to a disease the virulence of which is regulated by autoinducers. Also provided are methods of increasing disease resistance in susceptible plants and animals.

BACTERIAL STRAINS, GENES AND ENZYMES FOR CONTROL OF BACTERIAL DISEASES BY QUENCHING QUORUM-SENSING SIGNALS

FIELD OF THE INVENTION

The present invention relates to genes encoding regulators of bacterial metabolism, more particularly to genes encoding enzymes that quench quorum-sensing signals. The present invention further relates to methods of control of bacterial diseases comprising expression of genes encoding autoinducer inhibitors.

BACKGROUND OF THE INVENTION

N-acyl-homoserine lactones, known as autoinducers (AIs), are widely conserved signal molecules present in quorum-sensing systems of many Gram-negative bacteria. It has been found that AIs are involved in the regulation of a range of biological functions, including bioluminescence in *Vibrio* species (Eberhard et al., 1981; Cao and Meighen, 1989), Ti plasmid conjugal transfer in *Agrobacterium tumefaciens* (Zhang et al., 1993), induction of virulence genes in *Erwinia carotovora, Erw. chrysanthemi, Erw. stewartii, Pseudomonas aeruginosa, P. solanacerum,* and *Xenorhabdus nematophilus* (Jones et al., 1993; Passador et al., 1993; Pirhonen et al., 1993; Pearson et al., 1994; Beck von Bodman and Farrand, 1995; Flavier et al., 1998; Costa and Loper, 1997; Nasser et al., 1998;),

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regulation of antibiotic production in *P. aureofaciens* and *Erw. carotovora* (Costa and Loper, 1997; Pierson et al., 1994), regulation of swarming motility in *Serratia liquifaciens* (Eberl et al., 1996), and biofilm

5   formation in *P. fluorescens* and *P. aeruginosa* (Allison et al., 1998; Davies et al., 1998). Many more bacterial species are known to produce AIs, but the relevant biological functions have not yet been established (Bassler et al., 1997; Dumenyo et al.,

10   1998; Cha et al., 1998). Biofilm formation is of particular significance to bacterial pathogenicity, as it makes bacteria more resistant to antibiotics and host defense responses, and causes microbial contamination in medical devices and in drinking water

15   pipelines.

       Different bacterial species may produce different AIs. All AI derivatives share identical homoserine lactone moieties, but differ in the length and structure of their acyl groups. Although the target

20   genes regulated by AIs are extremely varied, the basic mechanism of AIs biosynthesis and gene regulation seems to be conserved in different bacteria. The general feature of gene regulation by AIs is cell density dependence, also known as quorum sensing. At low cell

25   densities the AIs are at low concentrations, and at high cell densities the AIs can accumulate to a concentration sufficient for activation of related regulatory genes (Fuqua and Winans, 1996). The biological functions regulated by AIs are of

30   considerable scientific, economic, and medical importance. New approaches for up or down regulation of bacterial quorum sensing systems would be of

significant value, not only in science, but also in practical applications.

It has been reported recently that a novel gene encoding autoinducer inactivation (*aiiA*) has been cloned from the Gram-positive bacterium *Bacillus* sp. strain 240B1 (Dong et al., 2000). Expression of the *aiiA* in transformed *Erw. carotovora* strain SCG1, a pathogen that causes soft rot disease in many plants, significantly reduces the release of AI, decreases extracellular pectrolytic enzyme activities, and attenuates pathogenicity on potato, eggplant, Chinese cabbage, carrot, celery, cauliflower, and tobacco. The results indicate the promising potential of using the AI-inactivation approach for prevention of diseases in which virulence is regulated by quorum sensing signals.

SUMMARY OF THE INVENTION

Bacterial strains and enzymes capable of efficient inactivation of N-acyl homoserine lactone autoinducers (AIs) are of considerable interest for biotechnology applications. With the present invention it is disclosed that all *Bacillus thuringiensis* strains and their closely related species tested were capable of enzymatic inactivation of AIs. One AI synthesis minus mutant of *Agrobacterium tumefaciens* strain A6, caused by Tn5 insertion mutagenesis, was also found capable of producing AI inactivation enzyme. The genes encoding for AI inactivation enzymes were cloned either by a functional cloning approach or by a PCR approach from the selected bacterial strains. A peptide sequence comparison indicates that all of these enzymes belong to the metallohydrolase family, with amino acid identity ranging from 35.4% - 94.0 % to the previously

reported AiiA enzyme. The *B. thuringiensis* strains
effectively quench AI activity when co-cultured with AI
producing pathogenic bacteria, and provide effective
biocontrol of potato soft rot disease caused by *Erwinia*
5   *carotovora*. The data suggest that quenching biosignals
which regulate virulence is an useful strategy for
disease control, and that *B. thuringiensis* strains
which are known for insecticidal activity are also
promising biocontrol agents for prevention of diseases
10   in which virulence is regulated by AIs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the time course of AI (OOHL)
inactivation by the protein extract of *A. tumefaciens*
strain M103. The total protein of M103 was extracted
15   by sonication disruption of bacterial cells in 1/15 M
phosphate buffer (pH 8.0). Equal volumes of M103
protein extract (1.46 mg/ml) and 5000 nM OOHL were
mixed and incubated in a 1.5 ml Eppendorf centrifuge
tube at 28°C. Same protein extract was denatured by
20   boiling for 5 min and used as a control. The samples
were taken after 1, 3, 6 hr after reaction and the
reaction was stopped by boiling for 3 min. The samples
were analyzed for AI activity.

Figure 2 shows the cloning of the AI inactivation
25   region from the cosmid clones of mutant M103. Two
cosmid clones were contained in cosmid vector pLAFR3
while the four sub-clones in plasmid vector pBluescript
II SK(+). Symbols: +, positive in AI inactivation; −,
negative in AI inactivation; E: *Eco*RI; P: *Pst*I.

30   Figure 3 shows (A) The potential ORFs in the 1.5
kb AI inactivation region predicted with a sequence
analysis program; and (B) Deletion analysis to define

the ORF encoding AI inactivation enzyme (AiiB). PCR
amplified fragments were cloned into vector pBluescript
II SK(+) (pBM clones) or in vector pKK223-3 (pKM
clones). The numbers under each clone indicate the
5   start and stop positions of the PCR fragments
corresponding to the nucleotide sequences of the 1.5 kb
region. All constructs were confirmed by sequencing
analysis. The start codon (GTG) and stop codon (TAA)
of the aiiB ORF are shown under the clone pKM103-315.·
10   Solid arrows indicate the location and direction of lac
and tac promoter in these clones, the ORFs were
indicated with open arrows. Symbols: +, positive AI
inactivation activity; -, negative AI inactivation
activity.
15   Figure 4 shows (A) the nucleotide sequence (SEQ ID
NO 1) and (B) predicted peptide sequence (SEQ ID NO 11)
of the aiiB gene cloned from A. tumefaciens M103. The
putative ribosome binding (SD) region and two PstI
restriction enzyme sites are underlined, and the
20   putative transcription termination codon is indicated.
           Figure 5 shows the protein sequence comparison of
AiiB (SEQ ID NO 11) and AttM (SEQ ID NO 21), a putative
protein encoded by the attM gene in the att region of
A. tumefaciens, but its biological function has not
25   been demonstrated experimentally (GenBank accession No.
U59485). These two proteins exhibit a high degree of
similarity (the center sequence represents the
consensus sequence, four fragments identical to amino
acids 8-43, 45-158, 160-186 and 188-263 of SEQ ID NO
30   11), but functional AiiB protein has an additional 7
amino acids in the N-terminus.
           Figure 6 shows a protein sequence comparison of

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AiiB (SEQ ID NO 11) and AiiA (SEQ ID NO 22), a putative metallohydrolase which inactivates AI cloned from *Bacillus sp.* 240B1. The two conserved zinc binding regions are underlined.

5   Figure 7 shows the functional cloning of the *aiiC* gene. (A) Enzymatic inactivation of AI by the suspension culture of Bt strain Cot1. Equal volume of cell suspension culture (OD_{600} = 1.1) and 40 µM OOHL were mixed and incubated at 28°C (▲). The boiled

10   culture and OOHL at same concentrations were used as control (■). The samples were taken at times as indicated for AI activity assay. (B) Direct subcloning of AI-inactivation regions from the cosmid clones pLAFR3-aiiC of *B. thuringiensis* Cot1. The cosmid clone

15   was digested by *Eco*RI and subcloned into the pGEM-7Z vector. The AI inactivation positive clone pGEM7-aiiC was identified by enzyme activity assay. The pGEM7-aiiC was further subcloned in the pBluescript II SK(+) vector after *Bam*HI digestion. The AI inactivation

20   region of about 1.4 kb in size contained in clone pBS-aiiC was completely sequenced. Restriction enzymes: E: *Eco*RI; B: *Bam*HI.

          Figure 8 shows (A) the nucleotide sequence (SEQ ID NO 2) and (B) predicted peptide sequence (SEQ ID NO 12)

25   of the *aiiC* gene cloned from the Bt strain Cot1. The nucleotide sequence of the *aiiC* ORF is indicated by the uppercase letters and the untranslated regions are indicated by the lower case letters.

          Figure 9 shows the nucleotide sequences and

30   predicted protein sequences of the genes *aiiD* (SEQ ID NOS 3 & 13), *aiiE* (SEQ ID NOS 4 & 14), *aiiF* (SEQ ID NOS 5 & 15), *aiiG* (SEQ ID NOS 6 & 16), *aiiH* (SEQ ID NOS 7 &

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17), *aiiI* (SEQ ID NOS 8 & 18), *aiiJ* (SEQ ID NOS 9 & 19)
and *aiiK* (SEQ ID NOS 10 & 20) from Bt strains B1, B2,
B17, B18, B20, B21, B22 and B25, respectively.

Figure 10 shows a phylogenetic tree analysis and
amino acid identity of 11 cloned AI inactivation genes.
The phylogenetic tree was produced by DNASTAR sequence
analysis software (DNASTAR Inc.). The distance is
shown below the tree. The amino acid identity of each
sample to AiiA is shown at the right hand of the graph.

Figure 11 shows the effect of Bt strains on AI
production by *Erwinia carotovora*. *Erw. carotovora* SCG1
was inoculated alone in 15 ml LB medium (♦) or co-
inoculated respectively in 1:1 ratio with Bt strains
Cot1 (Δ) and B1 (●), *E. coli* DH5α (▲) and *B.
fusiformis* (■) in the same medium. The inoculum
concentration (T_0) was 1 x 10^7 CFU/ml (colony forming
unit per milliliter) for SCG1 and 1 x 10^6 CFU/ml for
others. After incubation of 1, 2, 3, 4, 6 and 24 hours
at 30°C, the bacterial suspensions were taken and the
supernatants were used to bioassay the AI produced.
The data were means of four repeats.

Figure 12 shows the effect of Bt strain Cot1 on
control of potato soft rot disease caused by *Erw.
carotovora* SCG1. Dip: Potato slices were dipped into
suspensions of Bt strain Cot1 (C), *E. coil* DH5α (D) or
B. fusiformis (Bf) at a level of about 5 x 10^8 CFU/ml or
water (W) for about 20 sec and then dried in a sterile
air flow for about 20 min. The slices which showed no
moisture on the surface were inoculated with 2.5 μl of
bacterial suspension containing SCG1 cells equivalent
to 5 x 10^5 or 5 x 10^4 CFU. Mixture: the cell culture of
SCG1 (2 x 10^8 or 2 x 10^7 CFU/ml) was mixed respectively

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with equal volumes of Cot1 (C), *E. coil* DH5α (D), *B. fusiformis* (Bf) (5 x 10^8 CFU/ml) or water (W). Two point five microlitres of the mixture was inoculated onto the top of slices. The final cell numbers of SCG1

5   inoculated are 2.5 x 10^5 or 2.5 x 10^4 CFU, as marked in the second line below the graph. After 20 hours incubation at 28°C the maceration area was measured. The data were the means of 4 or 12 (12 for Cot1) repeats.

10   Figure 13 shows the influence of Bt strains Cot1 and B1 on the growth of *Erwinia carotovora* SCG1. *Erw. carotovora* SCG1 (■) was inoculated alone or coinoculated with Bt strain Cot1 (◆) and B1 (▲) respectively in a 1:1 ratio in 15-ml LB medium. Each

15   strain was inoculated to a final concentration of about 1 x 10^7 CFU/ml for SCG1 and 1 x 10^6 CFU/ml for the others in the T_0 medium. After 2, 4, 6 and 24 hours culture at 30°C, the bacterial suspensions were taken and diluted accordingly for spreading on plates for

20   colony counting. The experiment was repeated four times and mean data were presented. Top: SCG1, Cot1, and B1 were incubated and grown separately; Middle: SCG1 was co-incubated with Cot1; Bottom: SCG1 was co-incubated with B1.

25   Figure 14 shows changes in bacterial cell numbers (A) and development of soft rot symptom (B) on inoculated potato slices. Potato slices were dipped into Cot1 suspensions (5 x 10^8 CFU/ml) (▲) or water (◆) for about 20 sec and then dried in a Laminar Flow for

30   about 20 min. The slices were then inoculated with 5 μl of *Erw. carotovora* SCG1 (2 x 10^9 CFU/ml). After incubation of 1, 2, 3 and 4 days at 28°C, the

inoculated slices were cut into small pieces and 10 ml
of 0.1 M NaCl solution was added for resuspension of
bacterial cells. The mixture was shaken for 30 min and
the suspension was diluted accordingly and spread on to

5   plates for colony counting. The colony numbers of SCG1
were shown as log10 CFU/slice (▲ SCG1 only; ■ dipped
in Cot1) and the numbers of Cot1 (♦) as log10 CFU/mm^2.
The experiment was repeated four times and mean data
were presented.

10   DETAILED DESCRIPTION OF THE INVENTION
Ten genes encoding AI inactivation enzymes have
been cloned from 9 Gram positive bacterial isolates and
one Gram negative bacterium (*A. tumefaciens*). The
genes showed different levels of homologies to the *aiiA*

15   gene, which encodes a putative metallohydrolase with
strong AI inactivation activity (Dong et al., 2000).
Similar to AiiA, the zinc binding motif regions are
highly conserved in the enzyme proteins encoded by
these newly cloned AI inactivation genes. It is very

20   likely that these ten enzymes are also members of the
metallohydrolase family, and use the same molecular
mechanism as the AiiA for inactivation of N-acyl
homoserine lactone autoinducers. The present invention
further enriches the gene pool of AI inactivation

25   enzymes.
In *A. tumefaciens*, N-acyl homoserine lactone
autoinducers, mainly OOHL, are involved in regulation
of Ti plasmid conjugal transfer (Zhang et al., 1993).
The production of OOHL in *A. tumefaciens* is induced by

30   the conjugal opines secreted by crown gall tumours
(Zhang and Kerr, 1991). The OOHL in turn induces the
expression of *tra* genes. Tra proteins are responsible

for completing the process of Ti plasmid conjugal
transfer. Only a few hours are required from opine
induction to completion of Ti plasmid conjugal
transfer, so the Ti plasmid conjugal transfer can

5   therefore be regarded as only a transient event. One
embodiment of the present invention, the *aiiB* gene for
N-acyl homoserine lactone degradation, identified in *A.
tumefaciens*, highlights the possibility that the
bacterium has a sophisticated mechanism for control of

10   AI signal turn over. It is plausible that AI is
degraded in *Agrobacterium* after completion of the Ti
plasmid conjugal transfer.

       It has been noted that a majority of bacterial
isolates capable of AI inactivation are Gram positive,

15   belonging to *B. thuringenesis* and closely related
species. So far, most of the characterised quorum-
sensing signals in Gram-negative bacteria are N-acyl
homoserine lactones (Fuqua et al., 1996), while Gram-
positive bacteria produce oligopeptides as quorum-

20   sensing signals (Dunny and Leonard, 1997).

       Bacillus thuringiensis (Bt) has been used
extensively as a microbial insecticide during the last
30 years. The microorganism is a gram-positive, spore-
forming soil bacterium, and produces a crystalline

25   parasporal body consisting of one or more crystal (Cry)
proteins during sporulation, which shows biocidal
activity against insect families such as lepidopteran,
dipteran, and colepteran insects at larval stages
(Lambert and Peferoen, 1992). Some Bt strains have

30   also been reported to be active against other insect
families, as well as mites, nematodes, flatworms, and
protozoa (Feitelson et al., 1992). Different Bt

strains produce more than 28 different but related
groups of insecticidal crystal proteins
(http://www.biols.susx.ac.uk/ Home/Neil_
Crickmore/Bt/). Different groups of crystal proteins
5   are usually active against a specific spectrum of
insects, but do not affect other beneficial insects in
agriculture. Currently, Bt-based formulations are the
most widely used and most effective microbial
insecticides in agriculture.

10   As a valuable biocontrol agent, Bt has several
advantages including its specificity for target
insects, its low development cost, and its
environmental compatibility (Lambert and Peferoen,
1992). Bt is commonly found in natural soil, and
15   normally multiplies by cell division, but forms spores
when nutrients are depleted or when the environment
becomes adverse. These spores are highly resistant to
stress conditions such as heat and drought, enabling
the bacterium to survive periods of stress. This
20   sporulating Gram-positive micro-organism can be
formulated readily into stable products, such as a dry
powder, for insect or disease biocontrol. Bt also has
been subjected to many safety tests, with no harmful
effects for animals or human beings.

25   Bt has not been exploited for disease control
because it usually does not produce effective
antibiotics against bacteria and fungi. In the present
invention, it has been found that all tested Bt strains
are capable of inactivating AI, and that Bt strains
30   provide effective biocontrol against *Erw. carotovora*
infection, whereas *B. fusiformis* and *E. coli* strains
which do not have AI inactivation genes were unable to

provide biocontrol against *Erw. carotovora*. Bt strains
did not produce any antibiotics and were not inhibitory
to the growth of pathogen. The data strongly suggest
the important role of AI inactivation genes in disease

5   biocontrol. Because the AI diffuses easily into
bacterial cells, Bt, capable of eliminating AI
constantly from its surroundings, is a promising
biocontrol agent, not only for control of plant soft
rot disease caused by *Erw. carotovora*, but also for

10   control of other diseases in which the virulence genes
are regulated by AIs.

        Accordingly, an object of the present invention is
to provide a method for increasing resistance in a
plant or animal to a disease in which virulence is

15   regulated by AIs [such as the diseases caused by
*Pseudomonas aeruginosa, Erwinia stewartii, Erwinia
chrysanthemi, Pseudomonas solanacerum,* and *Xanthomonas
campestris* (Passador, *et al.*, 1993; Pirhonen, *et al.*,
1993; Pearson, *et al.*, 1994; Beck von Bodman and

20   Farrand, 1995; Barber, *et al.*, 1997; Clough, *et al.*,
1997; Costa and Loper, 1997; Nasser, *et al.*, 1998), and
especially plant soft rot disease caused by *Erw.
carotovora*] comprising administering to the plant or
animal an effective amount of a bacterium that is

25   capable of producing an autoinducer inhibitor. In a
preferred embodiment of this aspect of the invention,
the bacterium administered is a *Bacillus* sp., more
preferably a variety of *Bacillus thuringiensis*, most
preferably a variety of *B. thuringiensis* selected from

30   the group consisting of B1, B2, B17, B18, B20, B21, B22
and B25. In another preferred embodiment of this
aspect of the invention, the animal to be treated is a

human.

It is another object of the present invention to provide isolated nucleic acid molecules encoding autoinducer inactivation proteins. These nucleic acid
5   molecules encode autoinducer inactivation proteins that share the conserved amino acid motif
^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif
^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of these nucleic acid molecules encode the proteins of SEQ
10 ID NOS 11-20, and most preferred embodiments of these nucleic acid molecules have the sequences of SEQ ID NOS 1-10.

Another object of the present invention is to provide an expression vector that comprises at least
15 one nucleic acid sequence encoding an autoinducer inactivation protein, wherein the encoded protein comprises the conserved amino acid motif
^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif
^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, wherein the expression
20 vector propogates in a procaryotic or eucaryotic cell. Preferred embodiments of these expression vectors comprise at least one nucleic acid sequence encoding a protein having a sequence selected from the group consisting of SEQ ID NOS 11-20, and most preferred
25 embodiments have the nucleic acid sequences of SEQ ID NOs 1-10.

Yet another object of the present invention is to provide a cell of a procaryote or eucaryote transformed or transfected with an expression vector of the present
30 invention.

Yet another object of the present invention is to provide an isolated protein which has autoinducer

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inactivation activity, where the protein comprises the
conserved amino acid sequence
^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif
^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}.   Preferred embodiments of

5   the invention comprise proteins having the amino acid
sequences of SEQ ID NOS 11-20.

        Yet another object of the present invention is to
provide a method for increasing disease resistance in a
plant or animal, which method comprises introducing

10   into a cell of such plant or animal at least one
nucleic acid molecule that encodes an autoinducer
inactivation protein in a manner that allows said cell
to express said nucleic acid sequence, wherein said
autoinducer inactivation protein comprises the

15   conserved amino acid sequence
^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif
^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}.   Preferred embodiments of
this aspect of the invention comprise introducing at
least one nucleic acid molecule encoding a protein

20   having a sequence selected from the group consisting of
SEQ ID NOS 11-20, and most preferred embodiments
comprising introducing at least one nucleic acid
sequence selected from the group consisting of SEQ ID
NOS 1-10.

25   Yet another object of the present invention
relates to a method of preventing or reducing bacterial
damage to a plant or animal, which method comprises
administering to a plant or animal in need of such
prevention or reduction an effective amount of at least

30   one autoinducer inactivation protein, wherein said
protein comprises the conserved amino acid sequence
^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif

^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of this aspect of the invention comprise providing at least protein having the amino acid sequences of SEQ ID NOS 11-20.

5   Yet another object of the present invention relates to a method of preventing or reducing the formation of bacterial biofilms, which method comprises exposing biofilm-forming bacteria to at least one autoinducer inhibitor protein, wherein said protein
10   comprises the conserved amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, or the similar motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}. Preferred embodiments of this aspect of the invention comprise exposing the biofilm-forming bacteria to at least protein having the
15   amino acid sequences of SEQ ID NOS 11-20.

It is possible to further enhance the efficiency of Aii-producing bacterial strains by using a genetic approach to modify such strains, for example by introducing genes encoding for additional, or more
20   active, autoinducer inhibitors. It also is possible to optimise the enzyme activity of *aii* genes by an *in vitro* DNA evolution approach. Increasing the expression of Aii enzymes by coupling the *aii* gene to a strong promoter or increasing the copy number of the
25   *aii* gene in Bt cells would be another useful way to improve the capacity of Bt strains to quenching AI signals. It is likely that genetically modified Bt strains which secrete AI inactivation enzyme or contain the enzyme in the outer membrane of the cell could have
30   better efficiencies in quenching AI signals than their wild type parent strain. This is achievable by fusing an *aii* gene to a sequence encoding a secretion or a

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membrane attachment signal peptide.

The sequence may be introduced into plant or
animal cells by well-known methods. Methods for the
transformation or transfection of eukaryotic cells with
5   exogenous nucleic acid sequences include transfection,
projectile bombardment, electroporation or infection by
Agrobacterium tumefaciens. These methods are likewise
familiar to the person skilled in the area of molecular
biology and biotechnology and need not be explained
10   here in detail.

As pathogenic bacteria cells are confined to the
intercellular area of plant tissues, it is desirable to
target the Aii protein into the intercellular spaces.
Such may be accomplished by fusing a secretion signal
15   peptide to the Aii protein (Sato, *et al.*, 1995; Firek,
et al., 1993; Conrad and Fiedler, 1998; Borisjuk, *et
al.*, 1999). Alternatively, a plant membrane attachment
motif can be incorporated into the peptide sequence of
Aii for anchoring the Aii enzyme in the outer surface
20   of plant cell membrane.

The present invention also contemplates usage of a
bacterial autoinducer inactivation protein directly to
treat or prevent bacterial damage. For example, the
protein may be applied directly to plants in need of
25   such treatment or prevention. In a preferred
embodiment, the protein is applied in the form of a
composition which comprises an effective amount of the
protein and a suitable carrier. The composition may
have a wide variety of forms, including solutions,
30   powders, emulsions, dispersions, pastes, aerosols, *etc.*

The bacterial autoinducer inactivation protein may
also be used to treat bacterial infections in animals,

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including humans. In that application, an effective amount of the active ingredient is administered to an animal in need of such treatment.

5   For therapeutic treatment, the active ingredient may be formulated into a pharmaceutical composition, which may include, in addition to an effective amount of the active ingredient, pharmaceutically acceptable carriers, diluents, buffers, preservatives, surface active agents, and the like. Compositions may also

10   include one or more other active ingredients if necessary or desirable.

The pharmaceutical compositions of the present invention may be administered in a number of ways as will be apparent to one of ordinary skill in the art.

15   Administration may be done topically, orally, by inhalation, or parenterally, for example. Topical formulations may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Oral formulations include powders, granules,

20   suspensions or solution in water or non-aqueous media, capsules or tablets, for example. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be used as needed. Parenteral formulations may include sterile aqueous solutions which may also

25   contain buffers, diluents and other suitable additives. The dose regimen will depend on a number of factors which may readily be determined, such as severity and responsiveness of the condition to be treated.

Traditionally, microbial biocontrol has depended

30   on production of antibiotics or antimicrobial compounds (Cronin et al., 1997; Liao and Sapers, 1999; Emmert and Handelsman, 1999). The present invention offers an

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alternative strategy for biocontrol, based on quenching biosignals that are essential for virulence.

Example 1: Bacterial strains capable of inactivating autoinducers

5   To identify the genes responsible for inactivation of autoinducer signals, more than 400 field and plant bacterial isolates and about 100 stains of the laboratory bacterial culture collection were screened. The bacterial strains used to test the ability of

10   inactivating autoinducer signals were isolated from soil and plant suspensions as described previously (Dong et al., 2000), or obtained from *Bacillus* Genetic Stock Centre (BGSC) and the American Type Culture Collection (ATCC). *Erwinia carotovora* SCG1 was

15   isolated from Chinese cabbage leaves showing soft rot symptoms. It was confirmed by 16S DNA sequence and its characteristic production of autoinducer and induction of soft rot disease in potato and Chinese cabbage. These strains were grown at 28°C in Luria-Bertani (LB)

20   medium with shaking when necessary. *Agrobacterium tumefaciens* strains were grown at 28°C in YEB, in BM minimal medium (basic minimal nutrient added with mannitol as sole carbon source), or on nutrient agar plates (Difco Laboratories). Mannitol at a final

25   concentration of 0.2% was used as the sole carbon source in the minimal medium. *Escherichia coli* strains were grown at 37°C in LB or on LB agar plates. Antibiotics were added at the following concentrations, when required: rifampin at 50 μg/ml, streptomycin at

30   100 μg/ml, ampicilin at 100 μg/ml, kanamycin at 50 μg/ml, and tetracycline at 10μg/ml. X-gal (5-bromo-4-

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chloro-3-indolyl-B-D-galactopyranoside) (Promega) was included in media at 50 μg/ml for detection of β-galactosidase enzyme activity.

More than 30 strains showed different levels of AI
5   inactivation activity. To characterise the unknown isolates, the 16S rRNA sequences of these isolates were analysed by PCR amplification and subsequent sequencing. The sequence search showed the 16S rRNA sequences of those strains capable of inactivating AI
10   are highly homologous to that of *Bacillus thuringiensis* (Bt).

To test whether other *Bacillus* strains also have the AI-inactivation ability, known strains of *B. thuringenesis*, *B. cereus*, *B. mycoides,* and *B.*
15   *sphaericus* were selected for bioassay. For determination of the AI inactivation ability of bacterial strains and isolates, the autoinducer, N-β-oxo-hexanoyl-L-homoserine lactone (OHHL), or N-β-oxo-octanoyl-L-homoserine lactone (OOHL) was added to the
20   over-night bacterial cultures which were diluted to $OD_{600} = 1.1$, or to the protein extracts, at a final concentration of 20 μM, and incubated at 28 °C for 30 min. The AI remaining in the supernatant was then determined as previously described (Zhang, 1993; Dong
25   et al., 2000).

Table 1 shows the AI inactivation activities of the selected strains and some newly identified isolates. All the tested bacterial strains, except *B. sphaericus* and *B. fusiformis,* eliminated AI (at a
30   concentration of 20 μM OHHL) with different levels of enzyme activities. These strains include 13 known *Bacillus* species (strains starting with a "B" in Table

1), 1 known *Agrobacterium* and 9 *Bacillus* species identified by 16S rDNA sequence analysis. Among them, 12 bacterial strains showed a high level of AI-inactivation activity ($> 30 \ \mu M/h/OD_{600}$); 8 showed a medium level of activity ($25-30 \ \mu M/h/OD_{600}$); and the *A. tumefaciens* strain M103 showed a low level of activity ($4.5 \ \mu M/h/OD_{600}$). Except for *A. tumefaciens*, all these AI-inactivation strains are Gram-positive and belong to *B. thuringenesis* or its close related species.

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Table 1.   Bacterial strains and their AI-inactivation activity

	Strains	Source	Enzyme activity ($\mu M/h/OD_{600}$)
28-32	*Bacillus thuringiensis*	This work	32.4 ± 1.1
258-3	*Bacillus thuringiensis*	This work	32.5 ± 1.2
69	*Bacillus thuringiensis*	This work	30.9 ± 2.3
60-1	*Bacillus thuringiensis*	This work	28.2 ± 5.1
250	*Bacillus thuringiensis*	This work	23.4 ± 3.9
262	*Bacillus thuringiensis*	This work	23.1 ± 1.5
B18	*Bacillus thuringiensis*	This work	27.4 ± 3.0
B20	*Bacillus thuringiensis*	This work	32.7 ± 2.4
B21	*Bacillus thuringiensis*	This work	33.1 ± 0.8
B22	*B. thuringiensis ssp. kurstaki**	This work	32.8 ± 1.3
B23	*B. thuringiensis ssp. Israelensis**	BGSC(4Q7)	26.7 ± 3.5
B1	*B. thuringiensis ssp. thuringiensis*	BGSC (4A3)	32.5 ± 0.3
B2	*B. thuringiensis ssp. kurstaki*	BGSC (4D1)	33.0 ± 0.6
B12	*B. thuringiensis ssp. Aizawai*	BGSC (4J4)	33.5 ± 0.9
B17	*B. thuringiensis ssp. Wuhanensis*	Mycogen(PSS2A1)	28.8 ± 4.1
B25	*Bacillus cereus*	This work	33.7 ± 0.8
B14579	*Bacillus cereus*	ATCC (14579)	31.7 ± 0.6
B6462	*Bacillus mycoides*	ATCC (6462)	29.8 ± 2.2
240B	*Bacillus sp.*	This work	33.0 ± 1.0
Cot	*Bacillus thuringiensis*	This work	25.1 ± 2.4
M103	*Agrobacterium tumefaciens*	This work	4.5
269	*Bacillus fusiformis*	This work	0
B29	*Bacillus sphaericus*	BGSC (12A4)	0

*   Plasmid minus

**   Equal volume bacterial suspension (diluted to OD_{600} = 1.1 from overnight cultures) and OHHL (40 µM) were incubated at 28°C for 30 min and then OHHL remaining in the supernatant was determined as previously described (Zhang, 1993). The enzyme activity is shown as digested µM of OOHL per hour per OD_{600} of bacterial culture. Values represent mean ± standard deviation of 4 replicates. Strains starting with a "B" prefix are the known *Bacillus* species. Other *Bacillus* strains were identified by 16S rDNA sequence analysis.

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The evidence suggests that the AI-inactivation
gene is located in chromosomal DNA but not in a
plasmid, because Bt ssp. *kurstaki* strain B2 and its
plasmid minus derivative strain B22, both showed a
5   similar level of enzyme activity. The second plasmid
minus strain B23, belonging to *B. thuringenesis* ssp.
Israelensids, was also capable of enzymatic
inactivation of AI.

To investigate the genetic diversity of genes for
10   AI-inactivation, the representative bacterial strains
showing high, medium or low levels of AI-inactivation
activity were chosen for further cloning experiments.

Example 2:   Functional cloning of the *aiiB* gene from
Agrobacterium tumefaciens strain M103
15   The suicide plasmid pSUP10 (Simon et al, 1983) in
E. coli SM10 was used to introduce transposon Tn5
insertions into the genome of *A. tumefaciens* octopine
strain A6 by the protocol described by Garfinkel and
Nester (1980), except that the bacterial suspensions
20   were spread onto BM minimal plates containing kanamycin
(100 µg/ml). Total DNA of *A. tumefaciens* mutant strain
M103 was partially digested with *Eco*RI, the 20-30 kb
fragments were recovered from lower melting point
agarose gel and purified. The purified fragments were
25   ligated to the dephosphorized *Eco*RI site of the cosmid
vector pLAFR3 (Staskawicz et al., 1987). The ligation
mixture was packaged with GigapackTMIII XL Packaging
Extract (Stratagene) and then transfected into *E. coli*
DH5α. About 2000 individual colonies grown on the
30   selective medium containing tetracycline were
maintained as the genomic library of *A. tumefaciens*

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mutant strain M103. The cosmid clones containing Tn5
were selected on the medium containing kanamycin and
were further assayed for AI inactivation activity by
using the bioassay method described above. Subcloning

5   into the sequencing vector pGEM-7Zf(+) was carried out
by routine techniques (Sambrook et al., 1989).
Sequencing was performed on both strands by using the
ABI Prism dRhodamine Terminator Cycle Sequencing Ready
Reaction Kit (Perkin-Elmer Applied Biosystems).

10   *Agrobacterium tumefaciens* strain A6 produces N-
acyl homoserine lactone autoinducers (AI) which are
involved in regulation of Ti plasmid conjugal transfer
(Zhang and Kerr, 1991). But its derivative M103 caused
by Tn5 insertional mutagenesis is capable of

15   inactivation of AI. (Table 1 and Fig. 1). It is likely
that the gene encoding for AI degradation in strain A6
is regulated by a negative regulator, and the Tn5
insertion resulted in constitutive expression of the
gene for AI inactivation.

20   Based on the assumption that the AI inactivation
gene may be located downstream of the Tn5 insertion
site, the cosmid clones containing Tn5 transposon were
selected by the kanamycin resistance phenotype. Two
cosmid clones resistant to kanamycin and showing AI

25   inactivation activity were obtained from the cosmid
library of M103. Restriction analysis and bioassay
showed that a 5.2 kb *Eco*RI fragment conferred the AI
inactivation activity. Further subcloning narrowed
down the region to a 1.5 kb *Pst*I fragment (Fig. 2).

30   Sequence analysis showed that several putative open
reading frames (ORFs) starting with ATG or UTG were in
the fragment. One of the ORFs showed 96.8% identity in

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nucleotide sequence and 98% in amino acid sequence to
the *attM* gene (U59485) of *A. tumefaciens* identified
previously. However, AI inactivation activity was not
detected when expressing the *attM* in *E. coli* via an
5   expression vector pKK223-3. Deletion analysis of the
1.5 kb fragment showed that a 792bp ORF, its start
codon a GTG rather than the normal ATG, encoding for AI
inactivation (Fig. 3). The gene was named as *aiiB*
(Fig. 4). In comparison with the AttM whose biological
10   function has not been identified experimentally, the
AiiB has 7 extra amino acids at the N terminus (Fig.
5). AiiB showed 35.4% identity at the amino acid level
compared to the previously reported AiiA (Fig. 6).

Example 3:   Functional cloning of the *aiiC* gene from *B.*
15   *thuringinesis* strain Cot1
     The suspension culture of strain Cot1 eliminated
AI (20 µM) completely after 2 hr incubation, but
bacterial cells killed by boiling for 5 min failed to
inactivate AI (Fig. 7A), indicating an enzymatic
20   inactivation mechanism. To identify the gene encoding
for AI inactivation from Cot1, a cosmid library was
constructed by *Eco*RI partial digestion of the genomic
DNA of the bacterial isolate Cot1. Genomic DNA was
extracted from bacterial isolate Cot1 and digested
25   partially with *Eco*RI. The DNA fragments were ligated
to the dephosphorylized *Eco*RI site of cosmid vector
pLAFR3. Ligated DNA was packaged and transfected into
E. coli DH5α. Cosmid clones with AI inactivation
activity were identified by using the bioassay method
30   described above. Subcloning into the sequencing vector
pGEM-7Zf(+) or pBlueScript SK were carried out by

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routine techniques.

One clone showing AI inactivating function was identified from the one thousand cosmid clones screened. Restriction analysis showed that this clone contains an insert of 24 kb. All five fragments generated by *Eco*RI complete digestion were subcloned into pGEM7 vector. The bioassay of these subclones showed that one clone, pGEM7-aiiC with an insert of 5 kb, conferred the AI inactivation activity. Further subcloning identified a 1.4 kb *Bam*HI fragment contained in the clone pBS-AiiC which was responsible for the AI inactivation function (Fig. 7B). The complete sequence of the clone pBS-AiiC showed that there is an ORF of 750 bp nucleotides (from 166 to 918) which encodes a protein of 250 amino acids (Fig. 8). Cloning of this ORF in the *E. coli* expression vector confirmed that it encoded a functional AI inactivation enzyme, designated as AiiC. At the peptide sequence level, the AiiC gene showed 91% and 33% identity, to the AiiA and the AiiB respectively. The *aiiC* gene has no significant similarity to other known sequences in the databases by FASTA and BLAST analysis at either nucleotide or peptide levels.

Example 4: The autoinducer inactivation genes in Bt belong to the same gene family

Among the tested bacterial isolates with AI inactivation activity, all except the *A. tumefaciens* strain M103, are Gram positive, and belong to *B. thuringiensis* (Bt) or closely related bacterial species. The *aiiA* and *aiiC* genes from the two *Bacillus* strains showed a high level of similarity. It is very

likely that the *aiiA* and *aiiC* genes are highly
conserved among *B. thuringiensis* strains. DNA
hybridisation (Southern blot) analysis was performed
using an *aiiC* fragment as a probe. The genomic DNA was
5   isolated from 18 selected bacterial strains, B1 (Bt
ssp. *thuringiensis*), B2, B3 and B4 (Bt ssp. *kurstaki*),
B22 (Bt ssp. *kurstaki* plasmid minus), B12 (Bt ssp.
Aizawai), B16 and B17 (Bt ssp. *Wuhanensis*), B23 (Bt
ssp. *Israelensis*), and other Bt strains B18, B20, B21,
10   240B1, 471W, and Cot1 as well as B25 and B26 (*B.
cereus*), and B29 (*B. sphaericus*). Genomic DNA (20 µg)
digested with *Eco*RI was separated by electrophoresis in
0.8% agarose gel and then the DNA was transferred onto
Hybond-N+ membrane (Amersham Pharmacia, Biotech.)
15   according to manufacture's instructions. The 1.4 kb
*Bam*HI fragment containing the *aiiC* codon region was
labelled with DIG for use as a probe for hybridisation.
After hybridisation at 65°C, the membrane was washed
twice in 2x SSC, 0.1% SDS at room temperature for 5
20   min, followed by washing twice in 0.1x SSC, 0.1x SDS at
65°C for 15 minutes. After washing, the membrane was
detected with anti-DIG-AP conjugate, the NBT/BCIP
solution was used as colour substrate according to
manufacture's protocol (Boehringer Mannheim).
25   The result showed that one hybridising band was
clearly detected from all tested strains, except for
B29 (*B. sphaericus*). These results indicated that
there is a single gene, with sequence similar to *aiiC*,
present in all tested *B. thuringiensis* strains and its
30   closely related species *B. cereus*. This is in
agreement with the bioassay data (Table 1).

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Example 5:   Cloning of other AI inactivation genes from
more bacterial isolates

Since the genes for AI inactivation are highly
conserved, a PCR approach was used for the cloning of
5   other AI inactivation genes from the selected *B.*
thuringiensis isolates. Genomic DNA isolated from the
bacterial isolates B1, B2, B17, B18, B20, B21, B22 and
B25 was used as template. Primers were designed based
on the conserved sequences of the 5' and 3' ends of the
10   *aiiA* and *aiiC* gene. Standard PCR conditions were used
to amplify AI-inactivation genes from the selected
bacterial isolates. The primer sequences were: C5f:
5'- ATG GGA TCC ATG ACA GTA AAG AAG CTT TAT - 3'; C3r:
5'-GTC GAA TTC CTC AAC AAG ATA CTC CTA ATG -3'. The
15   PCR reactions were performed for 35 cycles of 30 sec at
94°C, 30 sec at 55°C and 1 min at 72°C using a Perkin
Elmer GenAmp PCR System 2400. Two separate PCR
reactions were performed to make sure there was no
error in the amplified sequences. The PCR products
20   were purified by using QIAquick PCR Purfication Kit
(QIAGEN) and the purified PCR fragment was ligated to
pGEM-T vector (Promega). Clones having inactivating
autoinducer activity were chosen for further study.
Two such clones from each strain were sequenced.
25   Nucleic acid sequence data and deduced amino acid
sequences were analysed with the DNASTARTM sequence
analysis software package (DNASTAR Inc.) and GCG
sequence analysis software (Genetics Computer Group,
Wisconsin). Database searches were performed using the
30   BLASTA search algorithm.

Fig. 9 shows the nucleotide and deduced peptide
sequences of 8 AI inactivation genes (named *aiiD* to

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aiiK) cloned from Bt strains B1, B2, B17, B18, B20, B21, B22 and B25 respectively. These sequences all contain an ORF of 750 bp, which encodes a protein of 250 amino acids.

5 Example 6: The autoinducer inactivation genes are highly conserved among members of Bt and closely related *Bacillus* spp.

Except for the *aiiB* gene, all other genes were cloned from the Gram positive bacterial isolates.

10 Sequence analysis indicates that the *aii* genes cloned from the Gram positive bacterial isolates are highly conserved, with high amino acid identities ranging from 90.4% to 94.0%, in comparison to that of AiiA (Fig. 10). The *aiiB* gene cloned from the Gram negative *A.*

15 *tumefaciens* showed less similarity to other *aii* genes and clustered as a single group in the phytogenetic tree (Fig. 10). These results indicate that the autoinducer inactivation genes are highly conserved among members of Bt and closely related *Bacillus*.

20 In these Aii protein sequences, all except AiiB contain several invariant histidines with glutamate residues showing a pattern of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa ~D^{191}; the AiiB of *A. tumefaciens* contains the similar, but distinct motif ^{103}HXHXDH^{108}~72aa~H^{180}~21aa ~D^{202}. This

25 pattern agrees with the metallohydrolase criterion (Vallee and Galdes, 1984). The motif HXHXDH in the *Arabidopsis* glyoxalase II was suggested to be involved in binding to zinc ion (Crowder et al., 1997). Site-directed mutagenesis has shown that all these residues

30 except the first histidine (^{104}H in AiiA) in this motif are necessary for AiiA activity. These invariant

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histidines and glutamate residues are also present in AiiB to AiiK, indicating they belong to the same group of autoinducer metallohydrolases.

Example 7: Effect of Bt strains on AI production by
5   Erwinia carotovora
        To test the effect of Bt strains on quenching AI production by pathogenic bacteria, Erw. carotovora SCG1 was co-cultured with Bt strains Cot1, B1, E. coil DH5α, and B. fusiformis respectively. AI was assayed as in
10   Example 1. The AI produced by strain SCG1 was detected after 2 hours incubation, and a rapid increase was observed from 2 to 6 hours incubation (for cell numbers, see Fig. 14), whereas no AI was detected in the culture supernatant of SCG1 co-cultured with either
15   Cot1 or B1 strain, which produce AI inactivation enzymes. In the co-culture supernatants of SCG1 with either E. coil DH5α or B. fusiformis, which do not contain aii genes, AI production levels were detected that were similar to those observed with SCG1 culture
20   alone (Fig. 11). These results indicate that Bt strains effectively quench AI signals produced by the pathogen Erw. carotovora SCG1 when the two are cultured together.

Example 8: Effect of Bt strains on the pathogenesis of
25   Erwinia carotovora
        It is known that AI play a key role in regulation of the virulence determinates of several pathogenic bacterial species. Since Bt strains effectively quenched AI signals produced by the pathogen, it is
30   likely this new function of Bt strains can be exploited

for disease control. To test this possibility, the
effect of Bt strains for biocontrol against plant soft
rot disease was investigated. Potato (*Solanum
tuberosum* L. cv. Bintje) tubers were obtained from

5   local stores. After rinsing in tap water and drying on
paper towel, potato tubers were surface-sterilized with
70% ethanol, and then were sliced evenly to a 3mm
thickness. For the dip treatment, the potato slices
were dipped into the bacterial suspension of Cot1, or

10   other bacterial strains, diluted to a concentration of
5×10^8 colony forming unit (CFU) per ml, for about 20
seconds. Sterilised water was used as a control. The
slices were dried in a laminar flow cabinet for about
20 min to remove surface moisture before inoculation

15   with 2.5 µl of *Erw. carotovora* SCG1 bacterial
suspension containing approximately 2×10^8 or 2×10^7,
CFU/ml onto the top of each slice. For the mixture
treatment, equal volumes of each testing organism ($5 \times
10^8$ CFU/ml), or sterile water were mixed with *Erw.*

20   *carotovora* SCG1 bacterial suspension (2×10^8 or 2×10^7
CFU/ml). The mixture (2.5 µl) was inoculated to a cut
surface of the potato slices. All the potato slices
were incubated in a Petri dish at 28°C. Maceration area
was measured during incubation. Each treatment was

25   repeated 4 to 12 time (12 for Cot1), each repeat was
inoculated 3 places on one slice. For the colonisation
experiment, each treatment was repeated 4 times, each
tuber slice was inoculated only once at the centre of
slice. Potato tuber slices were either treated with Bt

30   strain Cot1 or other controls first before inoculation
of *Erw. carotovora* SCG1, or SCG1 bacteria were mixed
with Cot1 or other controls before inoculation onto

potato slices.

Erw. carotovora SCG1 caused severe tissue
maceration of potato slices 20 hr after inoculation,
whereas on Bt strain Cot1 pre-treated potato slices the
5   maceration symptom was significantly attenuated (Fig.
12). Co-inoculation of SCG1 with the Bt strain Cot1
also attenuated soft rot symptoms, especially at the
lower concentration of inoculum. In contrast, control
treatments, either pretreatment of potato slices with
10   E. coli or B. fusiformis before inoculation of SCG1, or
co-inoculation of SCG1 with E. coli and B. fusiformis
respectively, showed severe tissue maceration symptoms
(Fig. 12). These results suggest that Bt strains could
be used as biocontrol agents against soft rot disease
15   in plants.

Example 9: In vitro competition between Bt strain and
Erwinia carotovora SCG1
        The Bt strains Cot1 and B1 were tested for
production antibiotics against Erw. carotovora SCG1.
20   Competition experiments were conducted by co-
inoculation of the Bt strain and Erw. carotovora in a
1:1 ratio. Each strain was inoculated at the level of
about 1 x 10^7 CFU/ml for Erw. carotovora and 1 x 10^6
CFU/ml for other strains. The mixture was incubated at
25   30°C. At different time points the bacteria samples
were taken for bioassay of AI production (the bioassay
performed as in Example 1), and were diluted in
suitable concentrations to spread on plates for colony
counting. The experiment was repeated four times. For
30   the colonisation experiment, the potato slices
inoculated with Erw. carotovora were taken at times as

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indicated, and plant tissues about 15 x 15 mm circling
the inoculation site were cut. The cut tissues were
cut into small piece and placed in 10 ml of 0.1M NaCl.
After shaking for 30 min, the supernatant was diluted

5   in suitable concentrations. Viable numbers of
bacterial cells were counted.

        On plates of both rich and minimum media, Bt
strains did not show any inhibitory effect on the
growth of SCG1. When strain SCG1 and Bt strain Cot1 or

10   B1 were coinoculated, both Bt strains and SCG1 grew
normally, showing the same growth trend over a 24 hr
period (Fig. 13).

Example 10:   Effect of Bt strain on colonisation of
tuber slice by *Erwinia carotovora*

15   To investigate colonisation of *Erw. carotovora*
SCG1 on potato slices after incubation, an expression
vector containing the GFP gene was transformed into
strain SCG1. The expression vector can be maintained
in strain SCG1 stably without selection pressure.

20   There was no difference in virulence between the SCG1
(GFP) and the wild-type SCG1. To investigate the
effect of Bt bacteria on the survival and growth of
SCG1 on plants, potato tuber slices were either dipped
into bacterial suspensions of Cot1, then inoculated

25   with SCG1(GFP), or simultaneously inoculated with
SCG1(GFP) and Cot1. Changes in bacterial cell numbers
and development of soft rotting symptoms of potato
tissue were monitored daily for 4 days. Results showed
that there were no big changes in cell numbers between

30   SCG1(GFP) on the Cot1-treated slices and the SCG1(GFP)
on the water-treated slices during 4-days incubation

(Fig. 14). The result indicates that Bt strain Cot1
did not significantly affect the growth of SCG1(GFP) on
the potato tube slices, suggesting that attenuation of
the virulence of *Erwinia* SCG1(GFP) by Bt strain Cot1

5   was not due to inhibition of SCG1(GFP) cell growth.

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We claim:

1. An isolated nucleic acid molecule encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

2. An isolated nucleic acid molecule of claim 1, wherein the encoded protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

3. An isolated nucleic acid molecule of claim 1, wherein the encoded protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

4. An isolated nucleic acid molecule of claim 3, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

5. A method for increasing in a susceptible plant or animal resistance to a disease in which virulence is regulated by autoinducers, comprising administering to the plant or animal an effective amount of a bacterium that is capable of producing an autoinducer inhibitor.

6. The method of claim 5, wherein the bacterium administered is a *Bacillus* sp.

7.   The method of claim 6, wherein the *Bacillus* sp. is a variety of *Bacillus thuringiensis*.

8.   The method of claim 7, wherein the *Bacillus thuringiensus* variety is selected from the group consisting of B1, B2, B17, B18, B20, B21, B22 and B25.

9.   The method of any of claims 5-8, wherein administration is to an animal.

10.   The method of claim 9, wherein the animal is a human.

11.   An expression vector comprising at least one nucleic acid sequence encoding for an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence selected from the group consisting of $^{104}HXHXDH^{109}$~60aa~H^{169}~21aa~D^{191} and $^{103}HXHXDH^{108}$~72aa~H^{180}~21aa~D^{202}, and wherein the expression vector propogates in a procaryotic or eukaryotic cell, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

12.   An expression vector according to claim 11, comprising at least one nucleic acid sequence encoding an autoinducer inactivation protein, wherein the encoded protein comprises the amino acid sequence $^{104}HXHXDH^{109}$~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

13.   An expression vector according to claim 11,

comprising at least one nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

14.   An expression vector according to claim 13, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

15.   A protein having autoinduction inactivation activity, where the protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and

5   ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

16.   A protein of claim 15, wherein the protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the protein does not consist of the amino acid sequence of

5   SEQ ID NO 22.

17.   A protein of claim 15, wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

18.   A method for increasing disease resistance in a plant or animal, which method comprises introducing into a cell of such plant or animal at least one nucleic acid sequence that encodes an autoinducer

5   inactivation protein, in a manner that allows said cell to express said nucleic acid sequence, wherein said

autoinducer inactivation protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and

10   ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

19.   A method according to claim 18, wherein the encoded protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid

5   sequence of SEQ ID NO 22.

20.   A method according to claim 18, wherein the at least one nucleic acid is selected from the group consisting of SEQ ID NOS 1-10.

21.   A method of reducing bacterial damage to a plant or animal, which method comprises administering to a plant or animal in need of such reduction an effective amount of an autoinducer inactivation

5   protein, wherein said protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, with the proviso that the protein does not consist of the amino acid sequence of

10   SEQ ID NO 22.

22.   A method according to claim 21, wherein the protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the protein does not consist of the amino acid sequence of

SEQ ID NO 22.

23. A method according to claim 21, wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

24. A method according to any of claims 21-23, wherein administration is to an animal.

25. A method according to claim 24, wherein the animal is a human.

26. A cell of a procaryote or eukaryote stably transformed with at least one nucleic acid molecule encoding an autoinducer inactivation protein, wherein the encoded protein comprises an amino acid sequence selected from the group consisting of ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191} and ^{103}HXHXDH^{108}~72aa~H^{180}~21aa~D^{202}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

27. A cell of claim, wherein the encoded protein comprises the amino acid sequence ^{104}HXHXDH^{109}~60aa~H^{169}~21aa~D^{191}, with the proviso that the encoded protein does not consist of the amino acid sequence of SEQ ID NO 22.

28. A cell of claim 26, wherein the encoded protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

29. A cell of claim 28, comprising a sequence selected from the group consisting of SEQ ID NOS 1-10.

30. A method of reducing the formation of bacterial biofilms, comprising exposing biofilm-forming bacteria to at least one autoinducer inhibitor protein, wherein said protein comprises an amino acid sequence selected from the group consisting of $^{104}HXHXDH^{109}\sim60aa\sim H^{169}\sim21aa\sim D^{191}$ and $^{103}HXHXDH^{108}\sim72aa\sim H^{180}\sim21aa\sim D^{202}$, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

31. A method of claim 30, wherein the protein comprises the amino acid sequence $^{104}HXHXDH^{109}\sim60aa\sim H^{169}\sim21aa\sim D^{191}$, with the proviso that the protein does not consist of the amino acid sequence of SEQ ID NO 22.

32. A method of claim 31, wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS 11-20.

Figure 1

Figure 2

Figure 3

A

*Pst*I   *Pst*I
5' └───┘ 3'
   1   1581

          ⇒
       93 269
              ⇒
           238 318
                    ⇒━━━━━━━━━━━━▷
                 336   1106

B

Clones	Activity	
pBM103-160	+	160 ————————————————— 1360
pBM103-280	+	230 ————————————— 1106
pBM103-230	+	280 ——————————— 1106
pBM103-296	+	296 ——————————— 1106
pKM103-310	+	310 —————————— 1106
pKM103-320	–	320 —————————— 1106
pKM103-330	–	330 ———————— 1106
pKM103-336	–	336 ———————— 1106
pKM103-315	+	GTG 315 ———————— 1106 TAA

p lac→

p tac→

⇒━━━━━━━━━━▷
aiiB ORF
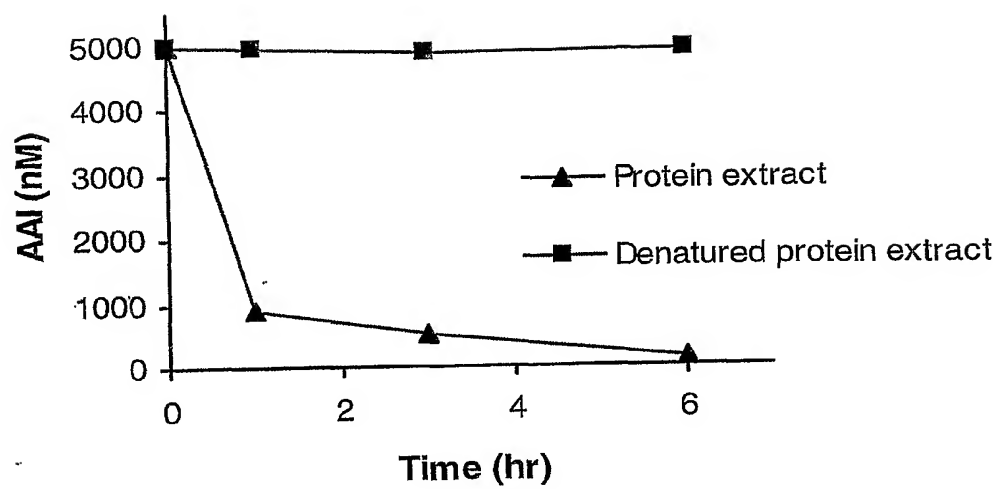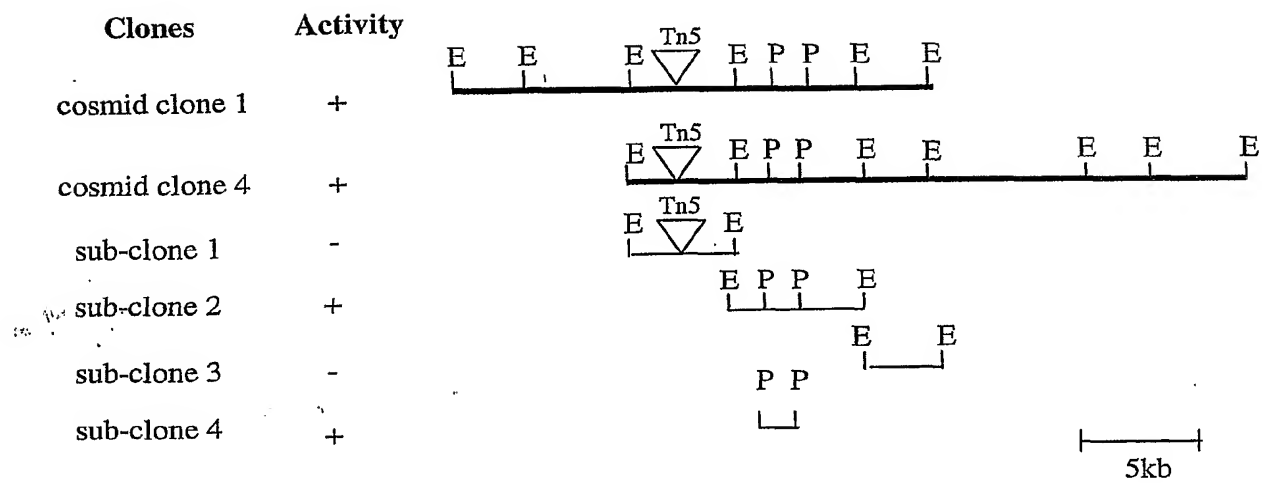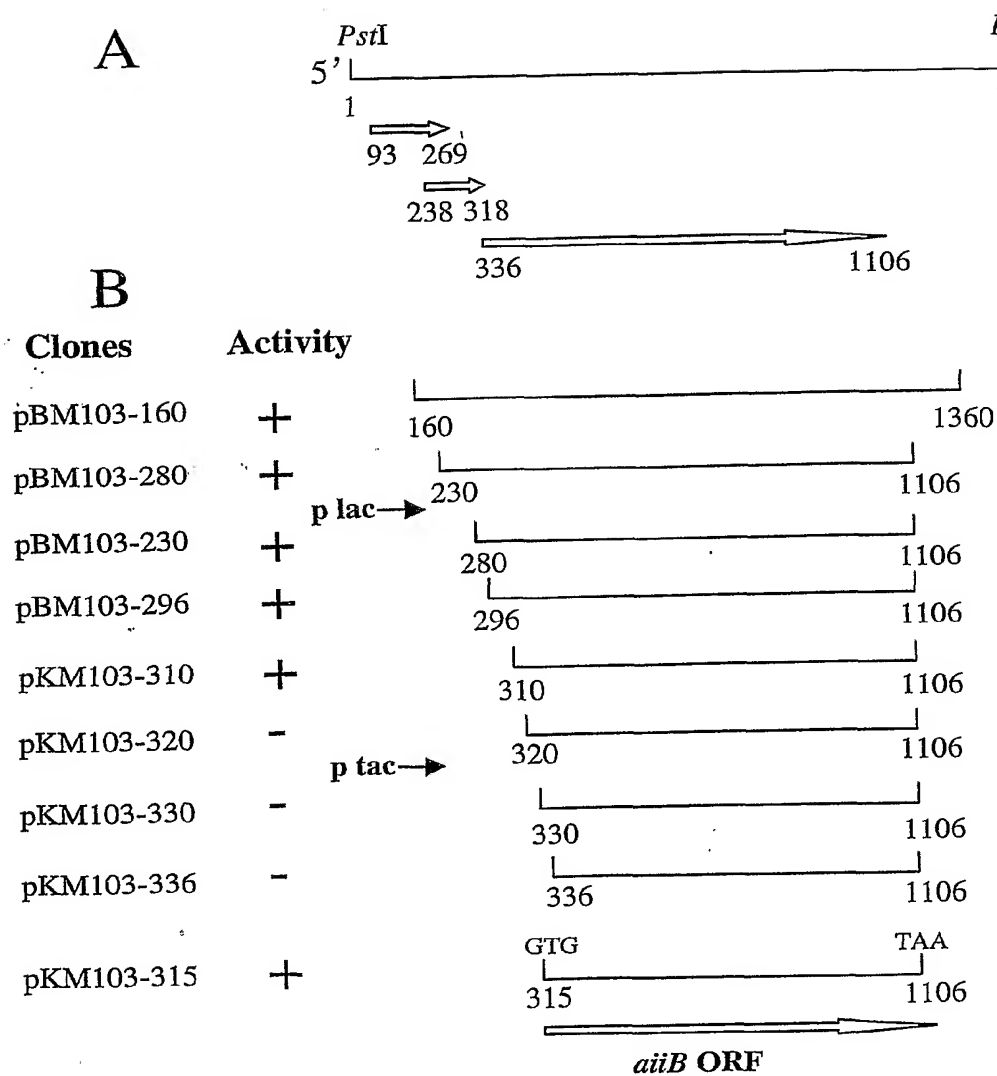
Figure 4

A

PstI
-314 *CTGCAG*CGTCGCTT -300

TATGCGGAGCTTGCCGACGTGCTGGGTGTTCCGGGTGAAGGGGATGCGGCAACCCGTTCG -240

GATGCGTTCGTTCAGCATATGGAAACGCTGATGGACGAAAGCGGCGCGCCGCGACGTCTG -180

CGCGATGTCGGCGTGACGGACAACACGCTCGCCATGCTTGCGTCCGACGCAATGAAACAG -120

AGCCGTCTGTTGGTCAATAATCCGGTCGAAGTCCGCGAAGAGGATGCGCTTGCGCTCTAC -60

CGCGAGGCGTTCTGACCCATTTCTGACAGCAATATCTTCAGTCCCAAGGG<u>AGGA</u>AAACGA -1
<u>SD</u>

<u>GTG</u>ACCGATATCAGACTTTACATGCTTCAGTCGGGTACGCTGAAATGCAAGGTACACAAC 60
start

ATCAAGATGAACCAGGGGAACGGTGCAGACTATGAGATCCCCGTTCCGTTTTTCCTGATT 120

ACCCATCCGGGCGGGCACACCGTGATCGACGGCGGCAACGCGATTGAAGTTGCAACGGAT 180

CCGCGTGGCCATTGGGGCGGCATCTGCGATGTCTATTGGCCAGTGCTGGACAAGGACCAG 240

GGCTGCGTTGACCAGATCAAGGCGCTTGGTTTCGATCCGGCCGATGTCAAGTATGTTGTG 300

CAGTCGCACCTGCATCTCGATCATACCGGCGCCATCGGTCGCTTCCCCAACGCAACCCAC 360

ATCGTGCAGCGCTCGGAATATGAGTATGCCTTCACGCCCGACTGGTTTGCCGGTGGCGGC 420

TATATCCGCAAGGACTTCGACAAGCCGGGCCTGAAGTGGCAGTTCCTCAACGGTACGCAG 480

GACGACTATTACGACGTTTACGGCGACGGCACGCTCACCACGATCTTCACGCCCGGTCAT 540

GCGCCCGGCCACCAGTCCTTGCTGGTGCGACTGCCAAACAGCAAACCGCTTCTCCTGACG 600

ATCGATGCTGCCTACACCCTGGACCACTGGGAGGAGAAGGCTTTGCCTGGCTTCCTCGCC 660

TCGACCGTTGACACGGTCCGTTCGGTTCAGAAACTCCGAACCTATGCCGAAAAGCATGAT 720

GCGACGGTCGTTACCGGCCATGACCCTGACGCGTGGGCGAACTTCAAGAAGGCTCCCGAA 780

TTTTACGCGT<u>AA</u>ATAAAACGCGCAAGTCAACAGCCAGATGCGGCGAGGTTGCGTGCAGCC 840
stop

TCGCCGATTTTTGTCATATGAGCCAAGGACCCCGAACCTGGCGGGACCGTGTATTTCTGC 900

GCAGAGGCCTTTTCAGGATATACGCCTTCACTCAGGTCGTTCGCGTTGTCGCCTCAAGGC 960

CTGAAAGCTGTCCTCCCGCTGCGCGAGTGTCCCCATATGCGGTTTATTACCCCGGCGTTA 1020

CTGTGGGCCATCAGGCTTCGGGCTGACAATTTGCAAATGCCGGATGGCTTAAAGTAGACT 1080

TGTCTCTTTGATCCAAGCCGTCGGCAAATGGTGCAGATTGTGGCGCCTATTTTGCGTTCC 1140

CAAGGCGTCGGGCCAGCCATGCCCCCCAAAACAGGCTTGCGAAAAACCGAAGCGGCTCGT 1200

TGAAACCCGCGCCGGCCAGCAATGAAACGACCTCGTCTTCCGATCGGGGTGGCTCTGCAC 1260

<u>CCTGCAG</u> 1267
PstI

B

VTDIRLYMLQSGTLKCKVHNIKMNQGNGADYEIPVPFFLI 40

THPGGHTVIDGGNAIEVATDPRGHWGGICDVYWPVLDKDQ 80

GCVDQIKALGFDPADVKYVVQSHLHLDHTGAIGRFPNATH 120

IVQRSEYEYAFTPDWFAGGGYIRKDFDKPGLKWQFLNGTQ 160

DDYYDVYGDGTLTTIFTPGHAPGHQSLLVRLPNSKPLLLT 200

IDAAYTLDHWEEKALPGFLASTVDTVRSVQKLRTYAEKHD 240

ATVVTGHDPDAWANFKKAPEFYA. 263

Figure 5

```
AiiB : 1                                                          VTDIRLY   7

AttM                                                             -------

AiiB : 8    MLQSGTLKCKVHNIKMNQGNGADYEIPVPFFLITHPGGHTVIDGGNAIEVATDPRGHWGG  67
            MLQSGTLKCKVHNIKMNQGNGADYEIPVPFFLITHP GHTVIDGGNAIEVATDPRGHWGG
AttM : 1    MLQSGTLKCKVHNIKMNQGNGADYEIPVPFFLITHPAGHTVIDGGNAIEVATDPRGHWGG  60

AiiB : 68   ICDVYWPVLDKDQGCVDQIKALGFDPADVKYVVQSHLHLDHTGAIGRFPNATHIVQRSEY  127
            ICDVYWPVLDKDQGCVDQIKALGFDPADVKYVVQSHLHLDHTGAIGRFPNATHIVQRSEY
AttM : 61   ICDVYWPVLDKDQGCVDQIKALGFDPADVKYVVQSHLHLDHTGAIGRFPNATHIVQRSEY  120

AiiB : 128  EYAFTPDWFAGGGYIRKDFDKPGLKWQFLNGTQDDYYDVYGDGTLTTIFTPGHAPGHQSL  187
            EYAFTPDWFAGGGYIRKDFDKPGLKWQFLNG QDDYYDVYGDGTLTTIFTPGHAPGHQS
AttM : 121  EYAFTPDWFAGGGYIRKDFDKPGLKWQFLNGAQDDYYDVYGDGTLTTIFTPGHAPGHQSF  180

AiiB : 188  LVRLPNSKPLLLTIDAAYTLDHWEEKALPGFLASTVDTVRSVQKLRTYAEKHDATVVTGH  247
            LVRLPNSKPLLLTIDAAYTLDHWEEKALPGFLASTVDTVRSVQKLRTYAEKHDATVVTGH
AttM : 181  LVRLPNSKPLLLTIDAAYTLDHWEEKALPGFLASTVDTVRSVQKLRTYAEKHDATVVTGH  240

AiiB : 248  DPDAWANFKKAPEFYA  263
            DPDAWANFKKAPEFYA
AttM : 241  DPDAWANFKKAPEFYA  256
```

Figure 6

```
      . T . . . L Y . . . . G . . . C . . . . . . . N . . . . . .   Consensus

 1    V T D I R L Y M L Q S G T L K C K V H N I K M N Q G N G A D   AiiB protein.PRO
 1    M T V K K L Y F V P A G - - R C M L D H S S V N S T L T P G   AiiA protein.PRO


      . . . . . P . . . . L . . . . . G . . . . D . G . . . E . A   Consensus

31    Y E I P V P F - - F L I T H P G G H T V I D G G N A I E V A   AiiB protein.PRO
29    E L L D L P V W C Y L L E T E E G P I L V D T G M P - E S A   AiiA protein.PRO


      . . . . G . . . G . . . . . . V . . P . . . . . . . . V . .   Consensus

59    T D P R G H W G G I - - - C D V Y W P V L D K D Q G C V D Q   AiiB protein.PRO
58    V N N E G L F N G T F V E G Q V L - P K M T E E D R I V N I   AiiA protein.PRO


      . K . . G . . P . D . . Y . . . S H L H . D H . G . . G . F   Consensus

86    I K A L G F D P A D V K Y V V Q S H L H L D H T G A I G R F   AiiB protein.PRO
87    L K R V G Y E P E D L L Y I I S S H L H F D H A G G N G A F   AiiA protein.PRO


      . N . . . I V Q R . E Y E . . . . . . . . . . . . Y . . K .   Consensus

116   P N A T H I V Q R S E Y E Y A F T P D W F A G G G Y I R K D   AiiB protein.PRO
117   I N T P I I V Q R A E Y E - - - A A Q - - H S E E Y L - K E   AiiA protein.PRO


      . . . P . L . . . . . . G . . . . . Y . V . . . . . L . . .   Consensus

146   F D K P G L K W Q F L N G T Q D D Y Y D V Y G D G T L T T I   AiiB protein.PRO
141   C I L P N L N Y K I I E G D - - - - Y E V V P G V Q L - - L   AiiA protein.PRO


      . T P G H . P G H Q S L L . . . . . S . P . L L T I D A . Y   Consensus

176   F T P G H A P G H Q S L L V R L P N S K P L L L T I D A A Y   AiiB protein.PRO
165   H T P G H T P G H Q S L L I E T E K S G P V L L T I D A S Y   AiiA protein.PRO


      T . . . . E . . . . . . G F . . . . . . . . . S . . . L . .   Consensus

206   T L D H W E E K A - L P G F L A S T V D T V R S V Q K L R T   AiiB protein.PRO
195   T K E N F E N E V P F A G F D S E L - - A L S S I K R L K E   AiiA protein.PRO


      . . . K . . . . V . . G H D . . . . . . . . . . . . . . P E   Consensus

235   Y A E K H D A T V V T G H D P D A W A N F K K A - - - - P E   AiiB protein.PRO
223   V V M K E K P I V F F G H D I E Q - - - - E R G C K V F P E   AiiA protein.PRO


      . Y . -                                                       Consensus

261   F Y A .                                                       AiiB protein.PRO
249   - Y I .                                                       AiiA protein.PRO
```

Figure 7

A

B

pLAFR3-aiiC
(24kb)

pGEM7-aiiC
(5kb, *Eco*RI)

PBS-aiiC
(1339 bp, *Bam*HI)
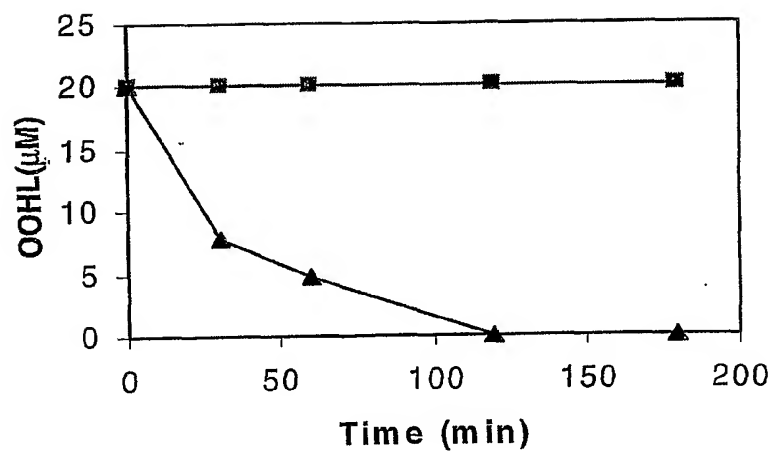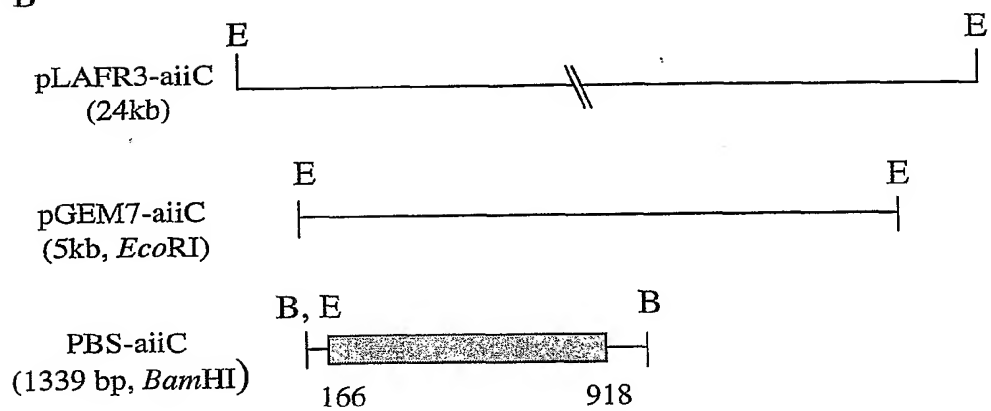
Figure 8

aiiC seq

A

gaattctttacttctatattatagatggtgaaatactgctatgtaaaaaaatacCctct	60
tttttctgtaagctgtactgatagtctagaaggagtttatttctaaaaagaagaattttt	120
tactgtattactttatcccaaactaaatgtaaaggtggatacataATGACAGTAAAGAAG	180
CTTTATTTCGTTCCAGCAGGTCGTTGTATGTTAGATCATTCTTCTGTTAATAGTACAATC	240
GCGCCGGGAAATTTATTGAACTTACCTGTATGGTGTTATCTTTTGGAGACGGAAGAAGGT	300
CCCATTTTAGTAGATACAGGTATGCCAGAAAGTGCGGTTAATAATGAAAACTTGTTTGAA	360
GGGACATTTGCAGAAGGACAGATTTTACCGAAAATGACTGAAGAAGATAGAATAATAGCT	420
ATTTTAAAACGTGCAGGGTATGAGCCAGATGACCTCCTATATATTATTAGTTCACATTTG	480
CATTTTGATCATGCAGGAGGAAATGGTGCTTTTATTAATACTCCAATCATTATACAGCGT	540
GCTGAATATGAGGCAGCGCAGTATAGAGAGGAATATTTGAAAGAGTGTATACTGCCGAAT	600
TTGAACTACAAAATTATTGAAGGGGATTATGAAGTGGTACCAGGTGTTCAACTATTGTAT	660
ACACCAGGACATTCACCAGGGCATCAGTCACTATTAATTGAGACAGAAAAATCTGGTGTT	720
GTGTTATTAACCATTGATGCATCTTATACGAAAGAGAATTTTGAAGATGAAGTACCGTTT	780
GCTGGATTTGATCCAGAATTAGCTTTATCATCAATTAAACGTTTAAAAGAAGTTGTGATG	840
AAAGAGAAGCCGCTTGTTTTCTTTGGACATGATATAGAGCAGGAAAAGGGATGTAAAGTG	900
TTCCCGGAATATATATAGtgcaaaaagtcatgagcttacgtgctcatgactttttgattt	960
aaataatttttttaaataagttataaacttttttggaactatcttcatttaattgatagt	1020
acgtaagatttacatcatcaggagtatcttgctgtgcaatcatcacttcgttactatgat	1080
gatcaactacccatatgaaatattttttataagtaccatcctcaaatgtaatccacatat	1140
cacaatctattaaatctgatccttcttcatctaatgttaattttcctttttttggccgtat	1200
tcatactgttaatgaatgtctttaattcatctgttttgcgagaaagatatcttttttttg	1260
ttttaatggactcgacatgtatatctttattcctgtttcccaaaaagacaggggggct	1320
catttggatccctttgagt	1339

B

MTVKKLYFVPAGRCMLDHSSVNSTIAPGNLLNLPVWCYLLETEEGPILVDTGMPESAVNN	60
ENLFEGTFAEGQILPKMTEEDRIIAILKRAGYEPDDLLYIISSHLHFDHAGGNGAFINTP	120
IIIQRAEYEAAQYREEYLKECILPNLNYKIIEGDYEVVPGVQLLYTPGHSPGHQSLLIET	180
EKSGVVLLTIDASYTKENFEDEVPFAGFDPELALSSIKRLKEVVMKEKPLVFFGHDIEQE	240
KGCKVFPEYI	250

Figure 9

aiiD

ATGACAGTAAAGAAGCTTTATTTCATCCCAGCAGGTCGTTGCATGTTGGATCATTCGTCTGTTAACAGTGCGTTAACACC
GGGGAAACTATTAAACTTGCCGGTGTGGTGTTATCTTTTGGAGACGGAAGAAGGTCCTATTTTAGTAGACACAGGTATGC
CAGAAAGTGCAGTTAATAATGAAGGGCTTTTTAACGGTACATTTGTTGAAGGACAGATCTTACCGAAAATGACTGAAGAA
GATAGAATCGTGAATATATTAAAGCGTGTGGGGTATGAGCCGGACGACCTTTTATATATTATTATTAGTTCTCACTTACATTT
TGATCATGCAGGAGGAAACGGTGCTTTTACAAATACACCAATTATTGTGCAGCGAACGGAATATGAGGCAGCACTTCATA
GAGAAGAATATATGAAAGAATGTATATTACCGCATTTGAACTACAAAATTATTGAAGGGGATTATGAAGTGGTACCAGGT
GTTCAATTATTGTATACGCCAGGTCATTCTCCAGGCCATCAGTCGCTATTCATTGAGACGGAGCAATCCGGTTCAGTTTT
ATTAATGATTGATGCATCGTACACGAAAGAGAATTTTGAAGATGAAGTGCCGTTCGCAGGATTTGATCCAGAATTAGCTT
TATCTTCAATTAAACGTTTAAAAGAAGTTGTGAAAAAAGAGAAACCAATTATTTTCTTTGGTCATGATACAGAGCAGGAA
AAGAGTTGTAGAGTGTTCCCGGAATATATATAG

MTVKKLYFIPAGRCMLDHSSVNSALTPGKLLNLPVWCYLLETEEGPILVDTGMPESAVNNEGLFNGTFVEGQILPKMTEE
DRIVNILKRVGYEPDDLLYIISSHLHFDHAGGNGAFTNTPIIVQRTEYEAALHREEYMKECILPHLNYKIIEGDYEVVPG
VQLLYTPGHSPGHQSLFIETEQSGSVLLMIDASYTKENFEDEVPFAGFDPELALSSIKRLKEVVKKEKPIIFFGHDTEQE
KSCRVFPEYI

aiiE

ATGACAGTAAAGAAGCTTTATTTCATCCCAGCAGGTCGTTGCATGTTGGATCATTCGTCTGTTAACAGTGCGTTAACACC
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Figure 9 (continued)

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                         Figure 9 (continued)

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Figure 10

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aiiH	91.2
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aiiD	90.8
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799.5

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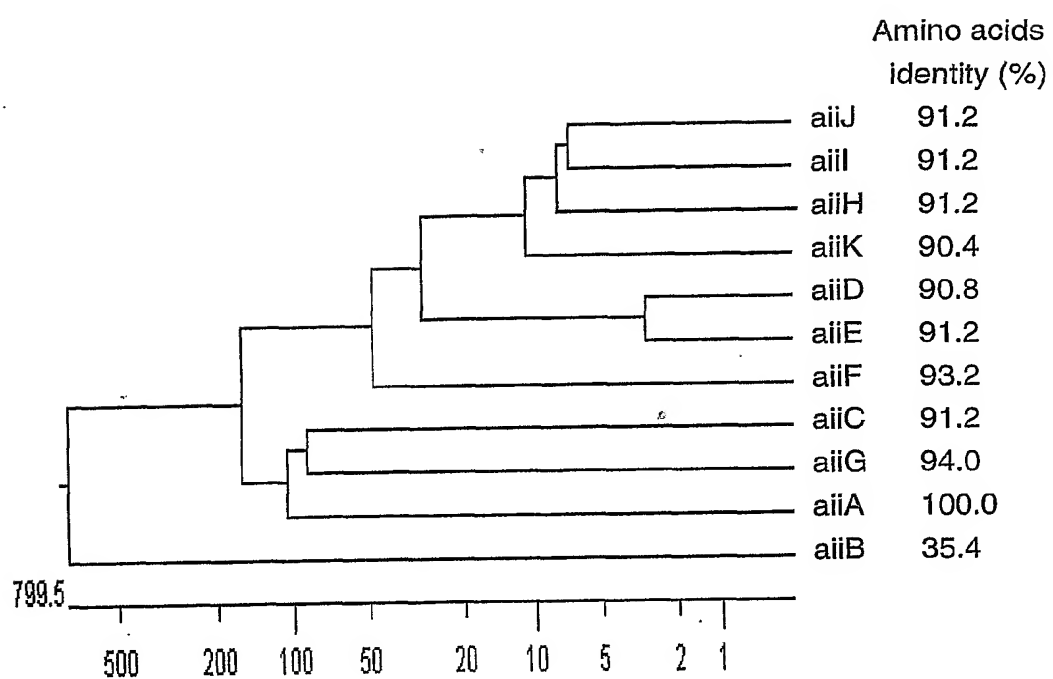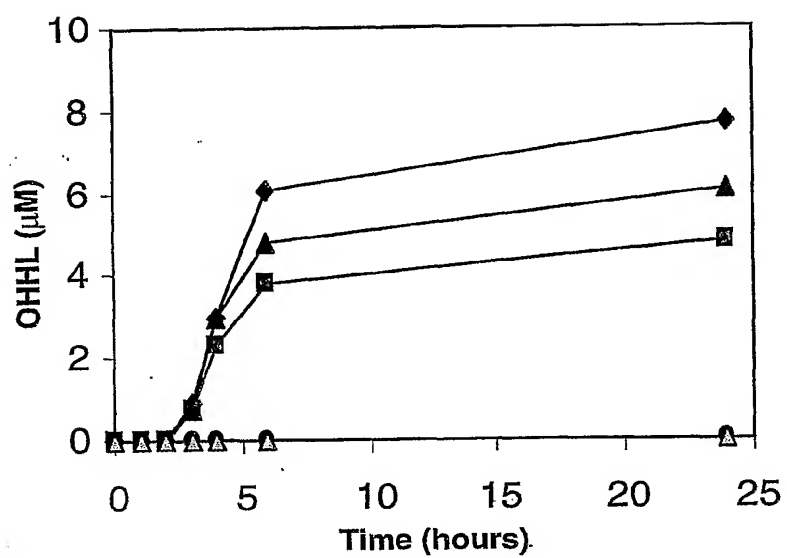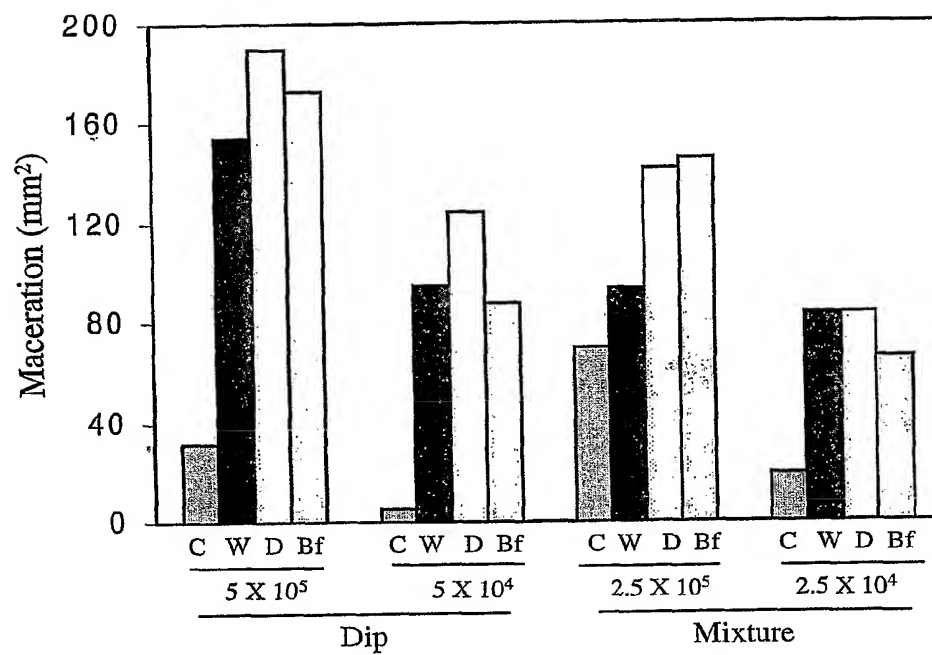
Figure 11

Figure 12

Figure 13
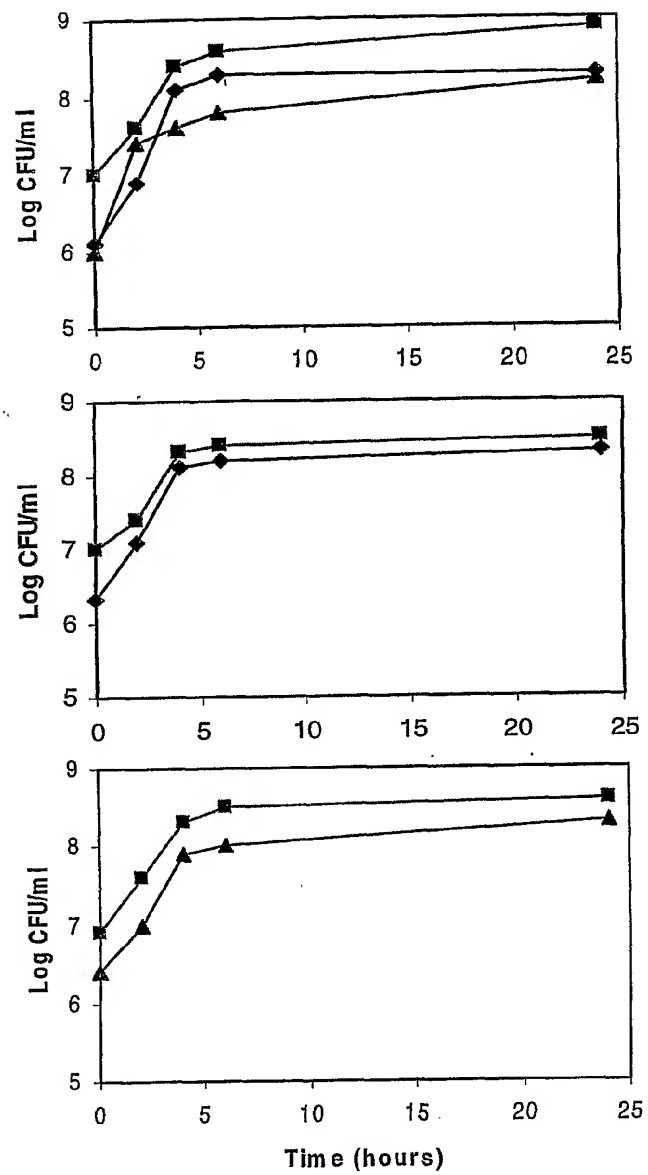
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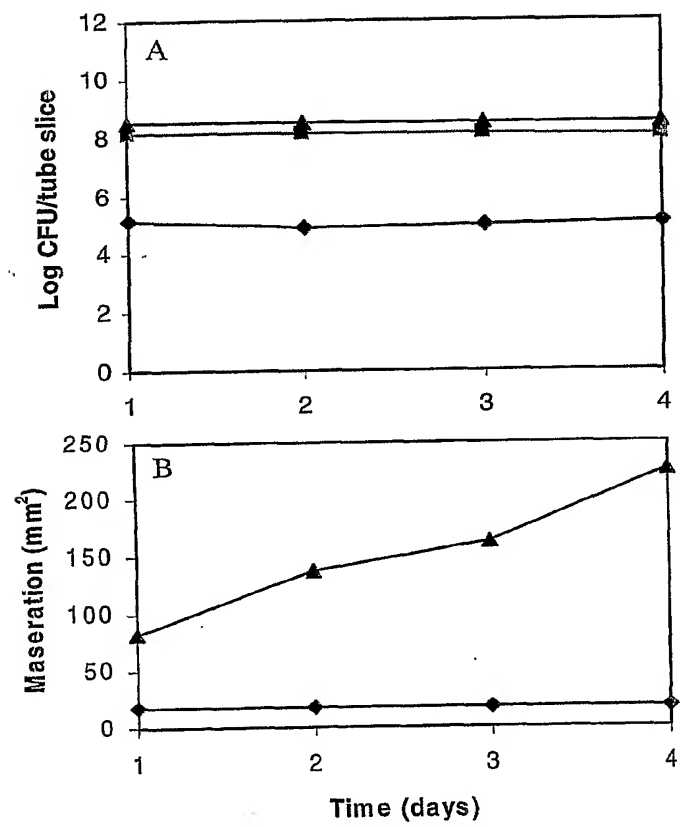
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gaaaaatccg gtcctgtatt attaacgatt gatgcatctt atacgaaaga gaattttgaa     600

gatgaagtac cgtttgcggg atttgattcg gaattagcct tatcttcaat taaacgttta     660

aaagaagttg tgatgaaaga gaaaccgatt gttttctttg gacatgatat agaacaggaa     720

aagggatgta aagtgttccc tgaatatata tag                                  753
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<210>   7
<211>   753
<212>   DNA
<213>   Bacillus thuringiensis B20

<400>   7
atgacagtaa agaagcttta tttcatccca gcaggtcgtt gtatgttaga tcattcttct      60

gttaatagta cactcgcgcc ggggaattta ttgaacttac ctgtatggtg ttatcttttg     120

gagacagaag aagggcctat tttagtagat acaggtatgc cagaaagtgc agttaataat     180

gaagggcttt ttaacggtac atttgttgaa ggacagattt taccgaaaat gactgaagaa     240
```

```
gatagaatcg tgaatatatt aaagcgtgta gggtatgagc cggacgacct tttatatatt    300

attagttctc acttacattt tgatcatgca ggaggaaacg gtgctttttac aaatacaccg    360

attattgtgc agcgagcgga atatgaggca gcacttcata gagaagaata tatgaaagaa    420

tgtatattac cgcatttgaa ctacaaaatt attgaagggg attatgaagt ggtaccaggt    480

gttcaattat tgtatacgcc aggtcattct ccaggccatc agtcgttatt cattgagacg    540

gagcaatccg gttcagtttt attaacaatt gatgcatcgt acacgaaaga gaattttgaa    600

gatgaagtgc cgttcgcagg atttgatcca gaattagctt tatcttcaat caaacgttta    660

aaaggagttg tggcggaaga gaaaccaatt gttttctttg gtcatgatat agagcaggaa    720

aagggttgta gagtgttccc tgagtatata tag                                 753
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<210>   8
<211>   753
<212>   DNA
<213>   Bacillus thuringiensis B21

<400>   8

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atgacagtaa agaagctttta tttcgtccca gcaggtcgtt gtatgttaga tcattcttct     60

gttaatagta cactcgcgcc ggggaattta ttgaacttac ctgtatggtg ttatctttttg    120

gagacagaag aggggcctat tttagtagat acaggtatgc cagaaagtgc agttaataat    180

gaagggcttt ttaacggtac atttgttgaa ggacagattt taccgaaaat gactgaagaa    240

gatagaatcg tgaatatatt aaagcgtgta gggtatgagc cggacgacct tttatatatt    300

attagttctc acttacattt tgatcatgca ggaggaaacg gtgctttttac aaatacaccg    360

attattgtgc agcgagcgga atatgaggca gcacttcata gagaagaata tatgaaagaa    420

tgtatattac cgcatttgaa ctacaaaatt attgaagggg attatgaagt ggtaccaggt    480

gttcaattat tgtatacgcc aggtcattct ccaggccatc agtcgttatt cattgagacg    540

gacaattccg gttcagtttt attaacaatt gatgcatcgt acacgaaaga gaattttgaa    600

gatgaagtgc cgttcgcagg atttgatcca gaattagctt tatcttcaat caaacgttta    660

aaaggagttg tggcggaaga gaaaccaatt gttttctttg gtcatgatat agagcaggaa    720

aagggttgta gagtgttccc tgagtatata tag                                 753
```

<210>   9
<211>   753
<212>   DNA
<213>   Bacillus thuringiensis B22

<400>   9

```
atgacagtaa agaagcttta tttcatccca gcaggtcgtt gtatgttaga tcattcttct        60

gttaatagta cactcgcgcc ggggaattta ttgaacttac ctgtatggtg ttatcttttg       120

gagacagaag aggggcctat tttagtagat acaggtatgc cagaaagtgc agttaataat       180

gaagggcttt ttaacggtac atttgttgaa ggacagattt taccgaaaat gactgaagaa       240

gatagaatcg tgaatatatt aaagcgtgta gggtatgagc cggacgacct tttatatatt       300

attagttctc acttacattt tgatcatgca ggaggaaacg gtgcttttac aaatacaccg       360

attattgtgc agcgagcgga atatgaggca gcacttcata gagaagaata tatgaaagaa       420

tgtatattac cgcatttgaa ctacaaaatt attgaagggg attatgaagt ggtaccaggt       480

gttcaattat tgtatacgcc aggtcattct ccaggccatc agtcgttatt cattgagacg       540

gagcaatccg gttcagtttt attaacaatt gatgcatcgt acacgaaaga gaattttgaa       600

gatgaagtgc cgttcgcagg atttgatcca gaattagctt tatcttcaat caaacgttta       660

aaaggagttg tggcggaaga gaaaccaatt gttttctttg gtcatgatat agagcaggaa       720

aagggttgta gagtgttccc tgagtatata tag                                   753
```

```
<210>    10
<211>    753
<212>    DNA
<213>    Bacillus thuringiensis B25

<400>    10
atgacagtaa agaagcttta tttcatccca gcaggtcgtt gtatgttaga tcattcttct        60

gttaatggta cactcgcgcc ggggaattta ttgaacttac ctgtatggtg ttatctttttg      120

gagacagaag aaggggccat tttagtagat acaggtatgc cagaaagtgc agttaataat       180

gaagggcttt ttaacggtac atttgttgaa ggacagattt taccgaaaat gactgaagaa       240

gatagaatcg tgaatatatt aaagcgtgta gggtatgagc cggacgacct tttatatatt       300

attagttctc acttacattt tgatcatgca ggaggaaacg gtgcttttac aaatacaccg       360

attattgtgc agcgaacgga atatgaggca gcacttcata gagaagaata tatgaaagaa       420

tgtatattac cgcatttgaa ctacaaaatt attgaagggg attatgaagt ggtaccaggt       480

gttcaattat tgtatacgcc aggtcattct ccaggccatc agtcgttatt cattgagacg       540

gagcaatccg gttcagtttt attaacaatt gatgcatcgt acacgaaaga gaattttgaa       600

gatgaagtgc cgttcgcagg atttgatcca gaattagctt tatcttcaat taaacgttta       660

aaaggagttg tggcgaaaga gaaaccaatt gttttctttg gtcatgatat agagcaggaa       720

aagggttgta gagtgttccc tgagtatata tag                                   753
```

<210> 11
<211> 263
<212> PRT
<213> Agrobacterium tumefaciens M103

<400> 11

Val Thr Asp Ile Arg Leu Tyr Met Leu Gln Ser Gly Thr Leu Lys Cys
1   5   10   15

Lys Val His Asn Ile Lys Met Asn Gln Gly Asn Gly Ala Asp Tyr Glu
            20   25   30

Ile Pro Val Pro Phe Phe Leu Ile Thr His Pro Gly Gly His Thr Val
        35   40   45

Ile Asp Gly Gly Asn Ala Ile Glu Val Ala Thr Asp Pro Arg Gly His
    50   55   60

Trp Gly Gly Ile Cys Asp Val Tyr Trp Pro Val Leu Asp Lys Asp Gln
65   70   75   80

Gly Cys Val Asp Gln Ile Lys Ala Leu Gly Phe Asp Pro Ala Asp Val
            85   90   95

Lys Tyr Val Val Gln Ser His Leu His Leu Asp His Thr Gly Ala Ile
            100   105   110

Gly Arg Phe Pro Asn Ala Thr His Ile Val Gln Arg Ser Glu Tyr Glu
        115   120   125

Tyr Ala Phe Thr Pro Asp Trp Phe Ala Gly Gly Gly Tyr Ile Arg Lys
    130   135   140

Asp Phe Asp Lys Pro Gly Leu Lys Trp Gln Phe Leu Asn Gly Thr Gln
145   150   155   160

Asp Asp Tyr Tyr Asp Val Tyr Gly Asp Gly Thr Leu Thr Thr Ile Phe
            165   170   175

Thr Pro Gly His Ala Pro Gly His Gln Ser Leu Leu Val Arg Leu Pro
            180   185   190

Asn Ser Lys Pro Leu Leu Leu Thr Ile Asp Ala Ala Tyr Thr Leu Asp
        195   200   205

His Trp Glu Glu Lys Ala Leu Pro Gly Phe Leu Ala Ser Thr Val Asp
    210   215   220

Thr Val Arg Ser Val Gln Lys Leu Arg Thr Tyr Ala Glu Lys His Asp
225   230   235   240

Ala Thr Val Val Thr Gly His Asp Pro Asp Ala Trp Ala Asn Phe Lys
            245   250   255

Lys Ala Pro Glu Phe Tyr Ala

260

```
<210>   12
<211>   250
<212>   PRT
<213>   Bacillus thuringiensis Cot1

<400>   12

Met Thr Val Lys Lys Leu Tyr Phe Val Pro Ala Gly Arg Cys Met Leu
1               5                   10                  15

Asp His Ser Ser Val Asn Ser Thr Ile Ala Pro Gly Asn Leu Leu Asn
            20                  25                  30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
        35                  40                  45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Asn Leu Phe
    50                  55                  60

Glu Gly Thr Phe Ala Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70                  75                  80

Asp Arg Ile Ile Ala Ile Leu Lys Arg Ala Gly Tyr Glu Pro Asp Asp
                85                  90                  95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100                 105                 110

Asn Gly Ala Phe Ile Asn Thr Pro Ile Ile Ile Gln Arg Ala Glu Tyr
        115                 120                 125

Glu Ala Ala Gln Tyr Arg Glu Glu Tyr Leu Lys Glu Cys Ile Leu Pro
    130                 135                 140

Asn Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145                 150                 155                 160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
            165                 170                 175

Leu Ile Glu Thr Glu Lys Ser Gly Val Val Leu Leu Thr Ile Asp Ala
            180                 185                 190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
        195                 200                 205

Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Glu Val Val
    210                 215                 220

Met Lys Glu Lys Pro Leu Val Phe Phe Gly His Asp Ile Glu Gln Glu
225                 230                 235                 240

Lys Gly Cys Lys Val Phe Pro Glu Tyr Ile
            245                 250

<210>   13
```

```
<211>    250
<212>    PRT
<213>    Bacillus thuringiensis B1

<400>    13

Met Thr Val Lys Lys Leu Tyr Phe Ile Pro Ala Gly Arg Cys Met Leu
1                5                   10                  15

Asp His Ser Ser Val Asn Ser Ala Leu Thr Pro Gly Lys Leu Leu Asn
            20                  25                  30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
        35                  40                  45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50                  55                  60

Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70                  75                  80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
                85                  90                  95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100                 105                 110

Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Thr Glu Tyr
        115                 120                 125

Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
    130                 135                 140

His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145                 150                 155                 160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
                165                 170                 175

Phe Ile Glu Thr Glu Gln Ser Gly Ser Val Leu Leu Met Ile Asp Ala
            180                 185                 190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
        195                 200                 205

Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Glu Val Val
    210                 215                 220

Lys Lys Glu Lys Pro Ile Ile Phe Phe Gly His Asp Thr Glu Gln Glu
225                 230                 235                 240

Lys Ser Cys Arg Val Phe Pro Glu Tyr Ile
                245                 250

<210>    14
<211>    250
<212>    PRT
<213>    Bacillus thuringiensis B2
```

Page 10

<400> 14

Met Thr Val Lys Lys Leu Tyr Phe Ile Pro Ala Gly Arg Cys Met Leu
1   5   10   15

Asp His Ser Ser Val Asn Ser Ala Leu Thr Pro Gly Lys Leu Leu Asn
          20   25   30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
          35   40   45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
      50   55   60

Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65   70   75   80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
              85   90   95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
          100   105   110

Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Thr Glu Tyr
          115   120   125

Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
      130   135   140

His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145   150   155   160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
          165   170   175

Phe Ile Glu Thr Glu Gln Ser Gly Ser Val Leu Leu Thr Ile Asp Ala
          180   185   190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
      195   200   205

Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Glu Val Val
      210   215   220

Lys Lys Glu Lys Pro Ile Ile Phe Phe Gly His Asp Ile Glu Gln Glu
225   230   235   240

Lys Ser Cys Arg Val Phe Pro Glu Tyr Ile
          245   250

<210>   15
<211>   250
<212>   PRT
<213>   Bacillus thuringiensis B17

<400>   15

```
Met Thr Val Lys Lys Leu Tyr Phe Val Pro Ala Gly Arg Cys Met Leu
1               5                   10                  15

Asp His Ser Ser Val Asn Ser Thr Leu Ala Pro Gly Asn Leu Leu Asn
            20                  25                  30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
        35                  40                  45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50                  55                  60

Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70                  75                  80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
                85                  90              .      95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100                 105                 110

Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Thr Glu Tyr
        115                 120                 125

Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
    130                 135                 140

His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145                 150                 155                 160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
            165                 170                 175

Leu Ile Glu Thr Glu Lys Ser Gly Leu Val Leu Leu Thr Ile Asp Ala
            180                 185                 190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
        195                 200                 205

Asp Ser Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Glu Val Val
    210                 215                 220

Met Lys Glu Lys Pro Ile Ile Phe Phe Gly His Asp Ile Glu Gln Glu
225                 230                 235                 240

Lys Gly Phe Lys Val Phe Pro Glu Tyr Ile
            .      245                 250
```

```
<210>   16
<211>   250
<212>   PRT
<213>   Bacillus thuringiensis B18

<400>   16
```

```
Met Thr Val Lys Lys Leu Tyr Phe Val Pro Ala Gly Arg Cys Met Leu
1               5                   10                  15
```

```
Asp His Ser Ser Val Asn Ser Ala Leu Thr Pro Gly Lys Leu Leu Asn
            20              25              30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
            35              40              45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50              55              60

Asn Gly Thr Phe Ala Lys Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65              70              75              80

Asp Arg Ile Val Thr Ile Leu Lys Arg Ala Gly Tyr Glu Pro Asp Asp
            85              90              95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100             105             110

Asn Gly Ala Phe Leu Asn Thr Pro Ile Ile Ile Gln Arg Ala Glu Tyr
    115             120             125

Glu Ala Ala Gln His Arg Glu Glu Tyr Leu Lys Glu Cys Ile Leu Pro
    130             135             140

Asp Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145             150             155             160

Val Arg Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
            165             170             175

Leu Ile Glu Thr Glu Lys Ser Gly Pro Val Leu Leu Thr Ile Asp Ala
            180             185             190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
    195             200             205

Asp Ser Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Glu Val Val
    210             215             220

Met Lys Glu Lys Pro Ile Val Phe Phe Gly His Asp Ile Glu Gln Glu
225             230             235             240

Lys Gly Cys Lys Val Phe Pro Glu Tyr Ile
            245             250

<210>   17
<211>   250
<212>   PRT
<213>   Bacillus thuringiensis B20

<400>   17

Met Thr Val Lys Lys Leu Tyr Phe Ile Pro Ala Gly Arg Cys Met Leu
1               5               10              15

Asp His Ser Ser Val Asn Ser Thr Leu Ala Pro Gly Asn Leu Leu Asn
            20              25              30
```

```
Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
        35                  40                  45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
        50                  55                  60

Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70                  75                  80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
                85                  90                  95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
                100                 105                 110

Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Ala Glu Tyr
        115                 120                 125

Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
        130                 135                 140

His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145                 150                 155                 160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
                165                 170                 175

Phe Ile Glu Thr Glu Gln Ser Gly Ser Val Leu Leu Thr Ile Asp Ala
                180                 185                 190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
        195                 200                 205

Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Gly Val Val
        210                 215                 220

Ala Glu Glu Lys Pro Ile Val Phe Phe Gly His Asp Ile Glu Gln Glu
225                 230                 235                 240

Lys Gly Cys Arg Val Phe Pro Glu Tyr Ile
                245                 250

<210>   18
<211>   250
<212>   PRT
<213>   Bacillus thuringiensis B21

<400>   18

Met Thr Val Lys Lys Leu Tyr Phe Val Pro Ala Gly Arg Cys Met Leu
1               5                   10                  15

Asp His Ser Ser Val Asn Ser Thr Leu Ala Pro Gly Asn Leu Leu Asn
                20                  25                  30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
        35                  40                  45
```

```
Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50                  55                  60

Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70                  75                  80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
                85                  90                  95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100                 105                 110

Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Ala Glu Tyr
            115                 120                 125

Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
    130                 135                 140

His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145                 150                 155                 160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
            165                 170                 175

Phe Ile Glu Thr Asp Asn Ser Gly Ser Val Leu Leu Thr Ile Asp Ala
            180                 185                 190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
            195                 200                 205

Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Gly Val Val
    210                 215                 220

Ala Glu Glu Lys Pro Ile Val Phe Phe Gly His Asp Ile Glu Gln Glu
225                 230                 235                 240

Lys Gly Cys Arg Val Phe Pro Glu Tyr Ile
                245                 250

<210>    19
<211>    250
<212>    PRT
<213>    Bacillus thuringiensis B22

<400>    19

Met Thr Val Lys Lys Leu Tyr Phe Ile Pro Ala Gly Arg Cys Met Leu
1               5                   10                  15

Asp His Ser Ser Val Asn Ser Thr Leu Ala Pro Gly Asn Leu Leu Asn
            20                  25                  30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
            35                  40                  45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50                  55                  60
```

```
Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70              75              80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
                85              90              95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100             105             110

Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Ala Glu Tyr
        115             120             125

Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
    130             135             140

His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145             150             155             160

Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
            165             170             175

Phe Ile Glu Thr Glu Gln Ser Gly Ser Val Leu Leu Thr Ile Asp Ala
            180             185             190

Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
        195             200             205

Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Gly Val Val
    210             215             220

Ala Glu Glu Lys Pro Ile Val Phe Phe Gly His Asp Ile Glu Gln Glu
225             230             235             240

Lys Gly Cys Arg Val Phe Pro Glu Tyr Ile
            245             250

<210>   20
<211>   250
<212>   PRT
<213>   Bacillus thuringiensis B25

<400>   20

Met Thr Val Lys Lys Leu Tyr Phe Ile Pro Ala Gly Arg Cys Met Leu
1               5               10              15

Asp His Ser Ser Val Asn Gly Thr Leu Ala Pro Gly Asn Leu Leu Asn
            20              25              30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Ala Ile Leu
        35              40              45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50              55              60

Asn Gly Thr Phe Val Glu Gly Gln Ile Leu Pro Lys Met Thr Glu Glu
65                  70              75              80
```

```
Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Asp Asp
            85                  90                  95
Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100             105             110
Asn Gly Ala Phe Thr Asn Thr Pro Ile Ile Val Gln Arg Thr Glu Tyr
        115             120             125
Glu Ala Ala Leu His Arg Glu Glu Tyr Met Lys Glu Cys Ile Leu Pro
    130             135             140
His Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145             150             155             160
Val Gln Leu Leu Tyr Thr Pro Gly His Ser Pro Gly His Gln Ser Leu
            165             170             175
Phe Ile Glu Thr Glu Gln Ser Gly Ser Val Leu Leu Thr Ile Asp Ala
            180             185             190
Ser Tyr Thr Lys Glu Asn Phe Glu Asp Glu Val Pro Phe Ala Gly Phe
        195             200             205
Asp Pro Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Gly Val Val
    210             215             220
Ala Lys Glu Lys Pro Ile Val Phe Phe Gly His Asp Ile Glu Gln Glu
225             230             235             240
Lys Gly Cys Arg Val Phe Pro Glu Tyr Ile
            245             250
```

```
<210>  21
<211>  256
<212>  PRT
<213>  Agrobacterium tumefaciens

<400>  21
```

```
Met Leu Gln Ser Gly Thr Leu Lys Cys Lys Val His Asn Ile Lys Met
1               5               10              15
Asn Gln Gly Asn Gly Ala Asp Tyr Glu Ile Pro Val Pro Phe Phe Leu
            20              25              30
Ile Thr His Pro Ala Gly His Thr Val Ile Asp Gly Gly Asn Ala Ile
        35              40              45
Glu Val Ala Thr Asp Pro Arg Gly His Trp Gly Gly Ile Cys Asp Val
    50              55              60
Tyr Trp Pro Val Leu Asp Lys Asp Gln Gly Cys Val Asp Gln Ile Lys
65              70              75              80
Ala Leu Gly Phe Asp Pro Ala Asp Val Lys Tyr Val Val Gln Ser His
            85              90              95
```

```
Leu His Leu Asp His Thr Gly Ala Ile Gly Arg Phe Pro Asn Ala Thr
            100                 105                 110

His Ile Val Gln Arg Ser Glu Tyr Glu Tyr Ala Phe Thr Pro Asp Trp
            115                 120                 125

Phe Ala Gly Gly Gly Tyr Ile Arg Lys Asp Phe Asp Lys Pro Gly Leu
    130                 135                 140

Lys Trp Gln Phe Leu Asn Gly Ala Gln Asp Asp Tyr Tyr Asp Val Tyr
145                 150                 155                 160

Gly Asp Gly Thr Leu Thr Thr Ile Phe Thr Pro Gly His Ala Pro Gly
                165                 170                 175

His Gln Ser Phe Leu Val Arg Leu Pro Asn Ser Lys Pro Leu Leu Leu
            180                 185                 190

Thr Ile Asp Ala Ala Tyr Thr Leu Asp His Trp Glu Glu Lys Ala Leu
            195                 200                 205

Pro Gly Phe Leu Ala Ser Thr Val Asp Thr Val Arg Ser Val Gln Lys
    210                 215                 220

Leu Arg Thr Tyr Ala Glu Lys His Asp Ala Thr Val Val Thr Gly His
225                 230                 235                 240

Asp Pro Asp Ala Trp Ala Asn Phe Lys Lys Ala Pro Glu Phe Tyr Ala
                245                 250                 255
```

```
<210>   22
<211>   248
<212>   PRT
<213>   Bacillus sp. 240B1

<400>   22
```

```
Met Thr Val Lys Lys Leu Tyr Phe Val Pro Ala Gly Arg Cys Met Leu
1               5                   10                  15

Asp His Ser Ser Val Asn Ser Thr Leu Thr Pro Gly Glu Leu Leu Asp
            20                  25                  30

Leu Pro Val Trp Cys Tyr Leu Leu Glu Thr Glu Glu Gly Pro Ile Leu
        35              40                  45

Val Asp Thr Gly Met Pro Glu Ser Ala Val Asn Asn Glu Gly Leu Phe
    50                  55                  60

Asn Gly Thr Phe Val Glu Gly Gln Val Leu Pro Lys Met Thr Glu Glu
65                  70                  75                  80

Asp Arg Ile Val Asn Ile Leu Lys Arg Val Gly Tyr Glu Pro Glu Asp
            85                  90                  95

Leu Leu Tyr Ile Ile Ser Ser His Leu His Phe Asp His Ala Gly Gly
            100                 105                 110
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Asn Gly Ala Phe Ile Asn Thr Pro Ile Ile Val Gln Arg Ala Glu Tyr
        115             120                 125

Glu Ala Ala Gln His Ser Glu Glu Tyr Leu Lys Glu Cys Ile Leu Pro
        130             135                 140

Asn Leu Asn Tyr Lys Ile Ile Glu Gly Asp Tyr Glu Val Val Pro Gly
145             150                 155                 160

Val Gln Leu Leu His Thr Pro Gly His Thr Pro Gly His Gln Ser Leu
            165                 170                 175

Leu Ile Glu Thr Glu Lys Ser Gly Pro Val Leu Leu Thr Ile Asp Ala
            180                 185                 190

Ser Tyr Thr Lys Glu Asn Phe Glu Asn Glu Val Pro Phe Ala Gly Phe
        195             200                 205

Asp Ser Glu Leu Ala Leu Ser Ser Ile Lys Arg Leu Lys Glu Val Val
    210             215                 220

Met Lys Glu Lys Pro Ile Val Phe Phe Gly His Asp Ile Glu Gln Glu
225             230                 235                 240

Arg Gly Cys Lys Val Phe Pro Glu
            245
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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C12N15/82   C07K14/195   A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C07K   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DONG YI-HU ET AL: "AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 97, no. 7, 28 March 2000 (2000-03-28), pages 3526-3531, XP002166712 March 28, 2000 ISSN: 0027-8424 cited in the application the whole document ─/──	1,2,5,6, 11,12, 15,16, 18,19, 21,22, 26,27,30

[X] Further documents are listed in the continuation of box C.   [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2001	22/05/2001

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Oderwald, H |

	Inte nal Application No
	PCT/SG 00/00123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMPRO2 'Online! EMBL; AC/ID U59485, 14 February 2000 (2000-02-14) MATTHYSSE A G ET AL.: "Agrobacterium tumefaciens AttM gene, required for attachment to host cells and virulence" XP002166713 see nucleotides 15.490 to 17.100 abstract —————	1,2,11, 12,15, 16,26,27

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-32 partially

An isolated nucleic acid molecule encoding an autoinducer inactivation protein (aiiB from Agrobacterium tumefaciens) comprising SEQ ID NO: 1. A protein encoded by said nucleic acid comprising SEQ ID NO: 11. Methods for increasing resistance to a disease, a method of reducing bacterial damage, a method of reducing the formation of bacterial biofilms, an expression vector, a host cell transformed with said nucleic acid.

2. Claims: 1-32 partially

same as invention 1 but comprising SEQ ID NO: 2-10 and 12-20 (aiiC to aiiK from Bacillus thuringiensis).